IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Group Art Unit: 1617
SCHASTEEN *et al.*	)	Examiner: S. Kantamneni
Application No.: 10/652,745	)	
Filed: August 29, 2003	)	Confirmation No. 1765
For: ANTIMICROBIAL COMPOSITIONS	)	

Attention: Mail Stop Appeal Brief-Patents
Commissioner for Patents
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Alexandria, VA 22313-1450

Sir:

REPLY BRIEF

In response to the Examiner's Answer mailed September 13, 2010, and further pursuant to 37 C.F.R. § 41.41, the Appellant presents this Reply Brief and hereby authorizes the Commissioner to charge any and all extensions or fees that may be required to Deposit Account No. 50-1662.

The following Reply Brief will clarify a number of issues before the Board. It will clarify that the Office relies upon the knowledge of a skilled artisan to make rejections under § 103, but has ignored the expert declaration of record attesting to the surprising and unexpected results of the currently claimed invention. It will clarify that the Office asserts each acid was "antimicrobial," but that the Office has ignored that the prior art specifically teaches away from a number of the acids with respect to what is actually claimed (killing or inhibiting *Salmonella*). It will also clarify that identifying which combinations of acids, and percentages of those acids, that would be effective at killing or inhibiting *Salmonella* was entirely unpredictable at the time of filing. It will also clarify that the currently claimed

invention is far superior to the prior art, a fact corroborated by the expert declaration of record, but has been not been considered by the Office.

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I. REAL PARTY IN INTEREST

Novus International, Inc., is the real party in interest, as indicated by the assignments in its name, recorded at Reel 015330, Frame 0531, and Reel 016014, Frame 0275.

II. RELATED APPEALS AND INTERFERENCES

The Appellant is unaware of any pending appeals or interferences that may directly affect or be directly affected by, or have a bearing on, the Board's decision in the pending appeal.

III. STATUS OF THE CLAIMS

Claims 75, 77-95, 114-117, 121-132, and 134-137 are pending in this application. Claims 1-74, 76, 96-113, 118-120, and 133 were previously canceled without prejudice. Claims 75, 77-95, 114-117, 121-132, and 134-137 have been finally rejected by the Examiner.

The Appellant hereby appeals the rejection of claims 75, 77-95, 114-117, 121-132, and 134-137. In accordance with 37 C.F.R. 41.37 (c)(1)(viii), a clean copy of the claims on appeal are set forth in full in the Claims Appendix to this brief.

IV. STATUS OF AMENDMENTS

No amendments to claims 75, 77-95, 114-117, 121-132, and 134-137 have been filed after the final rejection.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The present invention relates to methods for inhibiting or killing microbes in food, including human food, livestock food, pet food, or other animal food.[1] The present invention relates to treating food with an organic acid composition comprising at least three organic acids, the organic acid composition comprising

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[1] See, *e.g.*, specification at page 1, lines 10-26.

2-hydroxy-4-(methylthio)butanoic acid[2] and at least two organic acids[3] chosen from butyric acid,[4] lactic acid,[5] and propionic acid,[6] wherein the organic acid composition inhibits or kills more *Salmonella*[7] in the food compared to when the food is treated with any single organic acid that forms the organic acid composition.[8, 9, 10, 11]

Note: the currently claimed invention recites 2-hydroxy-4-(methylthio)butanoic acid, which is a compound of Formula (I).[12] The 2-hydroxy-4-(methylthio)butanoic acid is known and referred to in the art by various synonymous names including HMB, HMBA, HMTBA, and also under the proprietary trade name Alimet®. In the patent specification, the terms Alimet®, HMBA, HMTBA, and 2-hydroxy-4-(methylthio)butanoic acid are used interchangeably.[13]

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Claims 75, 77-87, 90-93, 115-117, 121-122, 124-125, 127-128, 130-131, and 134-137 stand rejected under 35 U.S.C. § 103(a) as obvious over Dunn *et al.* (U.S. Patent No. 4,824,686), Blake *et al.*

[2] See, *e.g.*, *id.*, at page 15, lines 16-28.
[3] See, *e.g.*, *id.*, at page 71, lines 1-4, page 72, lines 6-13 page 97, lines 23-25.
[4] See, *e.g.*, *id.*, at pages 106-125, including originally filed claims 3, 5, 7, 12, 13, 16, 17, 23-38, 43, 51, 59-63, 67, 70, 111, and 112. See also pages 63-105, including Examples 4-24.
[5] See, *e.g.*, *id.*, at pages 106-125, including originally filed claims 3, 5, 7, 12, 13, 16, 17, 23-38, 43, 51, 59-62, 64, 67, 70, 72, 73, 111, and 112. See also pages 63-105, including Examples 4-24.
[6] See, *e.g.*, *id.*, at pages 106-125, including originally filed claims 3, 5, 7, 12, 13, 16-22, 32-43, 51, 58, 60, 62-65, 67, 70, 72, 73, 111, and 112. See also pages 63-105, including Examples 4-24.
[7] See, *e.g.*, *id.*, at pages 65-83, including Examples 6-14.
[8] See, *e.g.*, *id.*, at Tables 11-20 at pages 71-83, including Examples 6-14.
[9] See, *e.g.*, *id.*, at page 8, lines 18-36, page 39, lines 14-32.
[10] See, *e.g.*, *id.*, at table 16 at page 78.
[11] See, *e.g.*, *id.*, at page 36, lines 30-36, page 37, lines 1-2, page 16, lines 1-5, page 35, lines 25-28.
[12] See, *e.g.*, *id.*, at originally filed claim 6
[13] See, *e.g.*, *id.*, at page 36, lines 5-8.

(U.S. Patent No. 2,938,053), Buttin (International Pig Topics), and Bland *et al.* (U.S. Patent No. 5,591,467).

B. Claims 88-89 stand rejected under 35 U.S.C. § 103(a) as obvious over Dunn *et al.*, Blake *et al.*, Buttin, Bland *et al.*, and Pinski *et al.* (U.S. Publication No. 2002/0172737).

C. Claims 94-95 stand rejected under 35 U.S.C. § 103(a) as obvious over Dunn *et al.*, Blake *et al.*, Buttin, Bland *et al.*, and Friedman *et al.* (U.S. Patent No. 4,495,208).

D. Claims 114, 123, 126, 129, and 132 stand rejected under 35 U.S.C. § 103(a) as obvious over Dunn *et al.*, Blake *et al.*, Buttin, Bland *et al.*, and Rolow *et al* (U.S. Patent No. 6,355,289).

VII. REPLY BRIEF ARGUMENTS

At the outset, the Appellant notes that no new grounds for rejection were asserted in the Examiner's Answer and therefore no new grounds of rejection must be addressed in the instant reply brief. The Appellant nevertheless hereby seeks to clarify the issues discussed in the Examiner's Answer before ultimate consideration by the Board.

Arguments Asserted by the Office Are Mistaken; Office Fails to Evaluate the Claimed Invention as a Whole or in view of Expert Declaration of Record

First, the Appellant respectfully submits that there are no § 102 rejections (*i.e.*, that the currently claimed combination is novel and the Office has found no novelty destroying references). At page 8, line 4, the Examiner's Answer asserts that physical properties are "inseparable from its composition," yet the Office has failed to show any identical composition or method in the prior art. Thus, the Office's assertion of inherency is erroneous. See MPEP 2112, "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may

not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999)." Here, the prior art and the Office acknowledge that the currently claimed invention is plainly different from the prior art, such that inherency is erroneous. See, for example, the Examiner's Answer at page 5, lines 13-18, wherein the Office admits that Dunn does not teach HMTBA, the prior art does not teach lactic acid and butyric acid, and the prior art does not teach the particular amounts of HMTBA, lactic acid, and butyric acid.

As a result of the Office's failure to evaluate the unique properties of the currently claimed invention, which are separate and distinct from the prior art, the Office has (1) has failed to rebut the synergism asserted by the Appellant with regard to the currently claimed invention, which is submitted to be novel form of synergism, and (2) has failed to explain how any synergism can be reliably predicted for a new combination of organic acids for the purposes of § 103. One of skill in the art would appreciate that new kinds of synergism exist, but which combinations are likely to exhibit synergism against *Salmonella* are highly unpredictable. The Examiner's assertions of "synergism" of other acid combinations by the prior art at page 5, line 1, and page 12, line 14, of the Examiner's Answer are respectfully submitted to be inaccurate and misleading. It is not the same synergism exhibited by the currently claimed invention. In addition, the Appellant has repeatedly asserted in the record that currently claimed invention is far superior to the prior art cited by the Office, a point which the Office has yet to address or acknowledge.

Second, the Appellant respectfully submits that an expert in the field has already provided his learned opinion on the record (attached to the previous Appeal Brief) that the organic acid formulations falling within the scope of the claims provide surprising and unexpected results. Although the Examiner would prefer more graphs and additional evidence, the expert's declaration stands on its own and the Office has done nothing to criticize his knowledge of the field or

to address his conclusions that the current invention is surprising and unexpected over the prior art. Importantly, the expert's conclusions were directed to the entire claimed invention's non-obviousness over the cited prior art and the remainder of technological field. The evidence of unexpected results is not limited to "mold inhibition" as alleged at page 19 of the Examiner's Answer or to the Figure attached to the declaration. In short, the Office is respectfully requested to consider the entire declaration along with the totality of the circumstances, which plainly support patentability.

Third, the Appellant respectfully submits that *Salmonella* is extremely difficult to kill in food, and that the prior art confirms this point. A number of the Office's cited references such as Bland *et al.* contain "key ingredients" (*e.g.* formaldehyde) which were excluded or omitted by the Examiner in making the current claim rejections, which undercut any motivation to combine or expectation of success in arriving at the currently claimed invention.

Fourth, the Office seems to maintain that all combinations of organic acids would have been predictable at killing *Salmonella*. However, the evidence of record and the expert declaration of record flatly contradict the Office's contention.

Fifth, the Appellant respectfully submits that the scope of the claims is consistent and commensurate with unexpected results and arguments asserted herein. The Appellant also submits that, in technology areas where there is a key question of fact (*i.e.*, the problem of killing *Salmonella* in feed) and nuances in the field are not fully appreciated by lay persons, that proper deference is granted to the expert in the field.

Sixth, the Office continues to assert that "antimicrobial activity" (See Examiner's Answer at page 6, line 13) is synonymous with "killing or inhibiting *Salmonella,*" but this is factually inaccurate. The Enthoven reference specifically recites that the same HMTBA cited by the Examiner's Answer at page 6, line 13, is ineffective at killing *Salmonella* (so too is propionic acid). The Office continues to ignore these express teachings away. One of skill in the art would appreciate

that there are a number of acids that are "antimicrobial," but are known to be ineffective at killing or inhibiting *Salmonella*. Thus, the "competence level of an ordinary skilled artisan" cited by the Examiner's Answer at lines 15-16 would actually support non-obviousness in the present case, because such a competence level would include the teachings away for acids known to be ineffective against *Salmonella*. As such, "antimicrobial" is not sufficient to provide a reasonable expectation of success regarding the currently claimed invention. Moreover, the Office fails to appreciate that adding additional acids known to be ineffective against Salmonella would likely decrease a composition's effectiveness against *Salmonella* – not increase it. These fundamental facts appear to be entirely ignored by the Office.

Seventh and finally, the Office has provided no rebuttal evidence against the fact that the Appellant's claimed invention is far superior to prior art compositions at killing or inhibiting *Salmonella*, which is submitted by the Appellant to be sufficient to overcome any *prima facie* case of obviousness.

Reassertion of Arguments in the Appeal Brief

The Examiner maintains the rejections of claims 75, 77-95, 114-117, 121-132, and 134-137. The arguments set forth below will address each basis of rejection under separate subheadings, in accordance with 37 C.F.R. 41.37(c)(1)(vii).

The Appellant will demonstrate herein that a *prima facie* case of obviousness has not been established or, alternatively, that any *prima facie* case of obviousness has been rebutted. Among other considerations, it will be shown that the cited prior art has been cited out of context and does not teach or suggest each and every element recited in the claims. When the prior art is considered in its entirety, it is apparent that there would have been no motivation to combine or reasonable expectation of success in combining the references in the manner cited. Importantly, the cited references include several portions that,

taken as a whole, lead away from the claimed invention and contradict a finding of obviousness.

It will also be shown that the methods of the claimed invention yield unexpected and superior results, which support a finding of non-obviousness. The results of the claimed methods are substantially greater than the additive effect of what would be expected from the sum of the individual components. The results of the claimed methods are also substantially greater than any of the individual components used at a proportionally equivalent volume. Evaluation of the data of record shows that the methods of the claimed invention inhibit or kill a substantially greater number of microbial colonies in food than otherwise would be expected. The evidence of record, including the expert declaration of Dr. Christopher Knight and Figure 7, further solidifies the unexpected results and nonobviousness of the currently claimed invention.[14] For the reasons detailed below, all pending claims are not rendered obvious by any combination of references as cited by the Office.[15]

For the purposes of this Appeal, claims 75, 77-95, 114-117, 121-132, and 134-137 do not stand or fall together. The claims have been divided into four groups: Group I (claims 75, 77-85, 88-95, and 134-137) Group II (claims 86 and 87); Group III (claims 114-117); and, Group IV (claims 121-132).

A. Summary of Claimed Invention; Substantial Improvement in Killing *Salmonella* in Food

[14] The Figure 7 identified in the Declaration shows that the claimed invention has approximately a 10-fold improvement or more over the prior art. The blends in Figure 7 are embodied by the currently claimed invention (*e.g.* claims 127 and 128 recite variations of Blend OA6).

[15] Nearly every patented invention is comprised of elements that previously existed in the prior art. "However, mere identification in the prior art of each element is insufficient to defeat the patentability of the combined subject matter as a whole." *In re Kahn*, 441 F.3d 977, 986 (Fed. Cir. 2006). Previously known components may be combined and arranged in new ways that were not previously foreseen or suggested, and which are patentable. As a result, precaution must be taken to avoid hindsight bias in evaluating whether a motivation to combine and a reasonable expectation of success existed at the time of filing.

As explained by the Appellant's specification and examples, the currently claimed invention is directed to a method comprising treating food with an organic acid composition comprising at least three organic acids, the organic acid composition comprising 2-hydroxy-4-(methylthio)butanoic acid and at least two organic acids chosen from butyric acid, lactic acid, and propionic acid, wherein the organic acid composition inhibits or kills more *Salmonella* in the food compared to when the food is treated with any single organic acid that forms the organic acid composition.

Of practical importance, the currently claimed invention offers a substantial improvement over the prior art and generally includes a substantial, exponential, and/or logarithmic improvement in killing *Salmonella* in food (*i.e.*, 10-fold or greater). The massive improvement in killing *Salmonella* in food is shown by the experimental results of record. See, *e.g.*, Tables 14-16 at pages 76-78 of the originally filed specification. Notably, the "Δ log reduction" in *Salmonella* in Table 17, at page 79 of the specification shows an improvement over the control group from 10-fold improvement to over 1000% in some of the experimental blends, noting that logarithmic improvement of 3.0 indicates an improvement of 10^3.0 = 1,000 times.

B. Unexpected Results Submitted by the Appellant; The Examiner's Failure to Consider Unexpected Results is Asserted to be Error in view of MPEP 2145

In addition to the experimental results shown in the specification, the Appellant has previously submitted additional objective evidence of non-obviousness, including the 37 C.F.R. § 1.132 declaration of Dr. Christopher Knight entered into the record, which states the currently claimed invention achieved **significantly greater killing of *Salmonella* than could be achieved with any of the individual organic acids alone**. An excerpt from item number four (4) from the declaration of Dr. Christopher Knight is provided below.

> 4. We have research data, that in my opinion, demonstrates surprising and unexpected results for organic acid formulations falling within the scope of the '434 patent claims. As an example, attached to this Declaration is a graph (identified as figure 7) that depicts a synergistic effect for two organic acid formulations of the claimed invention. With reference to the attached graph, data is depicted for the antimicrobial activity of five different organic acid compositions against *Salmonella* in feed. The five organic acid compositions include: (1) 0.45% HMTBA alone (i.e., 2-hydroxy-4-(methylthio)butanoic acid, which is a compound of Formula (I) in the '434 application); (2) 0.45% butyric acid alone; (3) 0.45% lactic acid alone; (4) blend OA 4, which is 0.15% lactic acid, 0.15% propionic acid, and 0.15% HMTBA; and (5) blend OA 6, which is 0.1% lactic acid, 0.1% butyric acid, 0.1% propionic acid, and 0.15% HMTBA. The antimicrobial experiments were conducted in accordance with Novus's standard protocol entitled "Low pH in Feed Test Procedure," a copy of which is attached to this Declaration. As depicted in the graph, the antimicrobial activity of either blend OA 4 or blend OA 6 achieved significantly higher killing of *Salmonella* at lower concentrations than could be achieved with any of the single organic acids alone.

The above-cited declaration was executed on September 25, 2007, and entered into the record on September 26, 2007, following the Examiner's non-final rejection on March 27, 2007. The objective evidence of non-obviousness, including the declaration and supporting figures and data, were resubmitted and explained numerous times to the Examiner, including September 26, 2007, April 11, 2008 (with reference to Figure 7), March 27, 2009, and also May 26, 2009.

The evidence, however, was improperly discounted and ignored by the Examiner. As stated by the Office in the Final Action mailed December 11, 2007, at page 16, lines 14-23, "The declaration under 37 CFR 1.1 32 filed by Dr. Christopher D. Knight is insufficient to overcome the rejection . . . is not convincing because no data is provided for the propionic acid alone for comparison." The Appellant asserts this is error by the Examiner, not only because the declaration itself states "**achieved significantly higher killing of *Salmonella* at lower concentrations than could be achieved with any of the single organic acids alone**," but also because the Examiner has ignored the experimental results in the original filed specification (*e.g.*, page 70, lines 25-27, "Combinations . . . **were compared to feed treated with propionic acid alone, and the results are shown in Figures 13-15.**"). (Emphasis added). See also

page 81, lines 5-9, which states, "antibacterial effect of two organic acid/Alimet blends were compared with blends containing formic and propionic acids, and with no Alimet following the procedure set forth in Example 12." More generally, the prior art of record also shows that propionic acid alone is ineffective at inhibiting *Salmonella* in food, and that deference should have been given to Dr. Knight's substantial knowledge of the industry. Finally, the Appellant also provided supporting data and graphs, including Figure 7, showing particular OA4 and OA6 blends of the current invention – again indicating surprising and unexpected results over the prior art or any of the individual organic acids alone.

The Examiner has thus far ignored the Appellant's evidence of unexpected results in violation of MPEP 2145. MPEP 2145 states, "When considering whether proffered evidence is commensurate in scope with the claimed invention, **Office personnel should not require the Appellant to show unexpected results over the entire range of properties possessed by a chemical compound or composition**. See, e.g., *In re Chupp*, 816 F.2d 643, 646, 2 USPQ2d 1437, 1439 (Fed. Cir. 1987). **Evidence that the compound or composition possesses superior and unexpected properties in one of a spectrum of common properties can be sufficient to rebut a prima facie case of obviousness**." (Emphasis Added). *Id.*

Thus far, the Office has failed to consider the totality of differences between Appellant's claimed invention and the prior art, including the expert declaration and other objective evidence of non-obviousness. The Appellant respectfully asserts that the currently claimed invention is non-obvious, unexpected, and superior over the prior art. Reversal is respectfully requested.

C. The Office Has Cited to Prior Art That Expressly Teaches Away and Indicates No Reasonable Expectation of Success; The Appellant Submits it Is Error for the Office to Ignore These Express Teachings Away

In the Office Action dated December 23, 2008, at page 4, lines 16-17, the Examiner asserted that "Enthoven et al. teaches that 2-hydroxy-4-

(methylthio)butanoic acid has antimicrobial effect." As such the Examiner asserted that a skilled artisan would allegedly be motivated to combine 2-hydroxy-4-(methylthio)butanoic acid **for the reason of antimicrobial effect**. Yet, the asserted motivation to combine is not rational or related to the claimed invention, which is specifically directed to inhibiting *Salmonella* in food. Enthoven expressly states that 2-hydroxy-4-(methylthio)butanoic acid (also referred to as HMB, HMBA, or HMTBA) has no inhibitory effect on *Salmonella*. Specifically, the Enthoven abstract discloses "the results show there is **no inhibitory effect of HMB** (2-hydroxy-4-(methylthio)butanoic acid) **or formic acid on *Lactobacillus* or *Salmonella***." Enthoven thus teaches away from the currently claimed invention – a method of killing or inhibiting *Salmonella* in food. As such, the **Examiner's asserted motivation to combine is flatly refuted by the reference to which the Examiner was citing**. Simultaneously, the Enthoven reference also establishes that there would be **no reasonable expectation of success to arrive at the currently claimed invention,** which is directed to inhibiting *Salmonella* in food. As such, the teachings away by Enthoven support the Appellant's own evidence of nonobviousness, including the data shown in Figure 7.

The Appellant asserts that it is error for the Examiner to ignore the teachings away by the prior art, particularly when they are consistent with the expert declaration and other evidence showing unexpected results and nonobviousness. Here, rather than weighing the evidence of nonobviousness provided by Enthoven, the Examiner merely stopped citing to the conflicting reference and appeared to ignore the objective evidence of nonobviousness that the reference provided. The Appellant respectfully asserts that **MPEP 2143.01(II) requires the Examiner to consider when one cited reference discredits or undercuts the basis for rejection**. MPEP 2143.01(II) states, "The test for obviousness is what the combined teachings of the references would have suggested to one of ordinary skill in the art, and all teachings in the prior art must be considered to the extent that they are in analogous arts. **Where**

the teachings of two or more prior art references conflict, the examiner must weigh the power of each reference to suggest solutions to one of ordinary skill in the art, considering the degree to which **one reference might accurately discredit another**. *In re Young*, 927 F.2d 588, 18 USPQ2d 1089 (Fed. Cir. 1991)." Here, the Appellant respectfully asserts that the Board consider the entirety of the prosecution record, including the Enthoven reference which provides teachings away, undercuts the Examiner's asserted motivation to combine, and refutes any reasonable expectation of success for the purpose of inhibiting *Salmonella* in food.

In addition, as provided by the MPEP, **references cannot be combined where a reference teaches away from their combination**. MPEP 2145(D)(2) states, "It is improper to combine references where the references teach away from their combination. *In re Grasselli*, 713 F.2d 731, 743, 218 USPQ 769, 779 (Fed. Cir. 1983) (The claimed catalyst which contained both iron and an alkali metal was not suggested by the combination of a reference which taught the interchangeability of antimony and alkali metal with the same beneficial result, combined with a reference expressly excluding antimony from, and adding iron to, a catalyst.)." In the present case, Enthoven states that neither HMTBA nor formic acid is effective at inhibiting *Salmonella*, but the Examiner is combining references exactly for that purpose – to kill *Salmonella* in food. Accordingly, the Examiner is citing references in violation of MPEP 2145. Nothing of record shows that HMTBA is effective against *Salmonella* in food, and the Examiner has thus far failed to identify a reason with a rational underpinning to make the alleged combination.

Whereas the Examiner does not utilize the Enthoven reference in making the current rejections under § 103, the Examiner fails to consider that Enthoven discredits and undercuts the current basis for rejection. The fact that Enthoven is prior art of record that expressly **teaches away** is highly relevant to the current issues under appeal. In particular, the Enthoven reference is respectfully

asserted by the Appellant as evidence that the rejection under § 103 is without merit. Reversal is respectfully requested.

D. The Examiner Has Not Shown a Reasonable Expectation of Success, as Required by MPEP 2143.02

The evidence of record, including the Enthoven reference discussed above, show that a number of organic acids are individually ineffective at inhibiting *Salmonella* in food. None of the references cited by the Examiner provide guidance as to which organic acids combinations would provide a reasonable expectation of success against *Salmonella* in food. None of the references, whether considered alone or collectively, provide guidance as to which organic acids combinations would provide the unexpected results against *Salmonella* in food that is achieved under the currently claimed invention.

To reject a claim under § 103, **MPEP 2143.02 requires a reasonable expectation of success to arrive at the currently claimed invention**. In light of the Supreme Court's instruction in *KSR*, the Federal Circuit has stated that, **"[t]o the extent an art is unpredictable, as the chemical arts often are, *KSR*'s focus on 'identified, predictable solutions' may present a difficult hurdle because potential solutions are less likely to be genuinely predictable**." *Eisai Co. Ltd. v. Dr. Reddy's Labs.*, Ltd, 533 F.3d 1353, 1359 (Fed. Cir. 2008). (Emphasis Added). Importantly, an obviousness determination requires that a skilled artisan would have perceived a reasonable expectation of success in making the invention in light of the prior art. In the present circumstance, the Examiner has failed to make an adequate or sufficient finding of fact regarding a reasonable expectation of success.

Moreover, since the Appellant has provided evidence of unexpected results and unpredictability, the threshold required to establish reasonableness is asserted to be higher for the currently pending claims. In the present case, the Examiner has failed to meet the reasonableness standard with regard to unpredictable arts. See *In re Kubin*, 561 F.3d 1351, 1360 (Fed. Cir. 2009).

MPEP 2144.08(e) states, "**If the technology is unpredictable**, it is less likely that structurally similar species will render a claimed species obvious because **it may not be reasonable to infer that they would share similar properties**. See, e.g., *In re May*, 574 F.2d 1082, 1094, 197 USPQ 601, 611 (CCPA 1978)." Here, in view of the foregoing (*e.g.*, Enthoven), the Appellant has shown there was considerable unpredictability in the prior art regarding antimicrobial compositions and the inhibition of *Salmonella* in food. Reversal is respectfully requested.

E. The Examiner Has Erred in View of MPEP 2144.09(V); Presumption of Obviousness Based on Structural Similarity is Overcome Where There is No Reasonable Expectation of Similar Properties; Reversal is Respectfully Requested

In the Final Action issued October 14, 2009, at page 5, lines 14-17, the Examiner states, "It would have been obvious to a person of ordinary skill in the art at the time of invention to add organic acids such as lactic acid, butyric acid to the preservative composition taught by Dunn et al. because Bland et al. teaches that lactic acid, butyric acid has antimicrobial activity." (Emphasis Added). The prosecution history, however, demonstrates that antimicrobial activity is unpredictable with respect to different microbes, including *Salmonella* in food. The Enthoven reference specifically undercuts the Examiner's assumption that all antimicrobials/antibiotics are effective against *Salmonella* in food. Accordingly, **the Examiner has failed to show a reasonable expectation of success against *Salmonella* in food – as specifically recited by the currently claimed invention**.

For the Examiner's broad interpretation of "antimicrobial activity " to be pertinent to the currently claimed invention, there would have to be a reasonable expectation of similar properties against *Salmonella* in food. In the present case, such a reasonable expectation of success has not been shown, has been refuted by the Appellant, and has been discredited by the prior art (*i.e.*, Enthoven). The

Appellant respectfully asserts that a *prima facie case* of obviousness has not been made.

MPEP 2144.09(V) recites that, "the presumption of obviousness based on structural similarity is overcome where there is no reasonable expectation of similar properties . . . See *In re May*, 574 F.2d 1082, 197 USPQ 601 (CCPA 1978) (appellant produced sufficient evidence to establish a substantial degree of unpredictability in the pertinent art area, and thereby rebutted the presumption that structurally similar compounds have similar properties); *In re Schechter*, 205 F.2d 185, 98 USPQ 144 (CCPA 1953). See also *Ex parte Blattner*, 2 USPQ2d 2047 (Bd. Pat. App. & Inter. 1987)." The facts in the present case are similar to those described in the MPEP, wherein there would be a substantial degree of unpredictability in the art. As such, the Examiner's presumption that all organic acids or antimicrobials have similar properties against *Salmonella* in food is mistaken and should be reversed.

MPEP 2144.09(VII) states, "A *prima facie* case of obviousness based on structural similarity is rebuttable by proof that the claimed compounds possess unexpectedly advantageous or superior properties. *In re Papesch*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963) . . . *In re Wiechert*, 370 F.2d 927, 152 USPQ 247 (CCPA 1967) (a 7-fold improvement of activity over the prior art held sufficient to rebut *prima facie* obviousness based on close structural similarity)." Thus, the MPEP recognizes that a 7-fold improvement of activity may be sufficient to rebut *prima facie* obviousness based on structural similarity. In the present case, the Appellant has submitted evidence of a 10-fold improvement or greater, which has thus far been ignored by the Office.

In the present case, the objective evidence of record shows substantial improvement, unexpected results, and superior properties over the prior art. The Examiner has cited no rebuttal evidence. Importantly, the Enthoven specifically undercuts the Examiner's assumption that all antimicrobials/antibiotics are effective against *Salmonella* in food. This undercuts the Examiner's contention

that all antimicrobials/antibiotics inherently perform this function or are predictable in this capacity. Reversal is respectfully requested.

F. The Rejection of Claims 75, 77-87, 90-93, 115-117, 121-122, 124-125, 127-128, 130-131, and 134-137 under 35 U.S.C. 103(a) over Dunn et al., Blake et al., Buttin, and Bland et al. is Improper

1. The Group I Claims Under Rejection (Claims 75, 77-85, 90-93, and 134-137)

Claim 75 is representative of the Group I claims. Claim 75 is directed to a method of inhibiting or killing *Salmonella* in food. The method comprises treating the food with an antimicrobial composition. The antimicrobial composition comprises 2-hydroxy-4-(methylthio)butanoic acid and at least two organic acids chosen from butyric acid, lactic acid, and propionic acid. The organic acid composition inhibits or kills more *Salmonella* in the food compared to when the food is treated with any single organic acid that forms the organic acid composition

The Final Action cites to Dunn et al., Blake et al., Buttin, and Bland et al. as allegedly disclosing one or more organic acids. Specifically, the Final Action at page 3, lines 7-9, asserts that "Dunn et al. teaches a method of killing microbes in animal feed such as pig feed, cattle feed, or poultry feed comprising treating animal feed with a binary blend of formic acid and propionic acid (preservative composition)." At page 4, lines 4-5, of the Final Action, the Examiner admits that, "Dunn et al. **do not teach** the employment of 2-hydroxy-4-(methylthio)butanoic acid in the preservative compositions therein." (Emphasis Added). The Examiner also admits at page 4, lines 6-7, "The prior art references **do not specifically teach the employment of organic acids such as lactic acid, butyric acid**." Finally, the Examiner admits at page 4, lines 8-9, "The prior art references **do not teach the particular amounts of 2-hydroxy-4-(methylthio)butanoic acid, lactic acid, butyric acid**. Despite these failings in the prior art, the Examiner asserts that, "Blake et al., teaches that Alimet, 2-

hydroxy-4-(methylthio)butanoic acid has antimicrobial activity, antifungal activity and thus on mixing Alimet (2-hydroxy-4- (methylthio)butanoic acid) with food kills microbes." Buttin et al. is further asserted to teach that in addition to providing a methionine source to food pH and provide relatively strong acid effect with a pKa of 3.6 (formic acid pKa = 3.75). Bland et al. is finally asserted to teach that organic acids such as formic acid, propionic acid, butyric acid, lactic acid have antibacterial properties and kill bacteria **<u>in solution</u>**. (Emphasis Added).

The alleged motivation to combine the cited references, as provided by the Examiner at page 5 of the Final Action is asserted as follows: "It would have been obvious to a person of ordinary skill in the art at the time of invention to **<u>add 2-hydroxy-4-(methy1thio)butanoic acid to the preservative composition taught by Dunn</u>** et al. **<u>because Blake et al.</u>**, teaches that 2-hydroxy-4-(methylthio)butanoic acid **<u>is an effective nutrient in poultry feed</u>**, and Blake et al., Buttin et al. teaches that 2-hydroxy-4-(methylthio)butanoic acid **<u>has antimicrobial activity</u>**."

a. The Examiner Fails to Make a *Prima Facie* Case of Unpatentability under § 103; All Claim Limitations Not Taught or Suggested

Collectively, the Examiner has cited to references that show organic acids being generally antimicrobial, without regard to which combinations, if any, are successful at inhibiting a specific microbe, *Salmonella* in food. The currently claimed methods require using "2-hydroxy-4-(methylthio)butanoic acid **<u>and at least two organic acids chosen from butyric acid, lactic acid, and propionic acid," for inhibiting or killing *Salmonella* in food</u>**. Although two organic acids from this group are required, the Examiner admits that, "**<u>The prior art references do not specifically teach the employment of organic acids such as lactic acid, butyric acid</u>**."

In the Final Action at page 6, lines 21-22, and page 7, lines 1-2, the Examiner asserts that, "Further, it is pointed out that Dunn et al. teach that the

mixture of formic acid, and propionic acid is a more potent inhibitor of salmonella infections than formic acid alone i.e. mixtures of organic acids is more potent than using a single organic acid." (Emphasis Added). The Appellant respectfully object to this comparison and conclusion by the Examiner because **formic acid has no inhibitory effect against *Salmonella* – it is a false comparison.** Per Enthoven, "[T]he results show there is **no inhibitory effect** of HMB (2-hydroxy-4-(methylthio)butanoic acid) or formic acid on *Lactobacillus* or *Salmonella*." **It is not obvious to combine acids which are thought to have no inhibitory effect against *Salmonella* for the purpose of inhibiting *Salmonella* in food.** None of the references cited by the Examiner indicate that HMBA, formic acid, lactic acid, or butyric acid is effective against *Salmonella* in food, as required by the currently pending claims. In fact, the Enthoven reference, as discussed above, teaches away from the Examiner's assumption that all organic acids have the same properties with regard to *Salmonella*.

As an additional matter, the Examiner has made no finding whatsoever regarding the claim limitation that the "composition inhibits or kills more *Salmonella* in the food compared to when the food is treated with any single organic acid that forms the organic acid composition." The Examiner has also repeatedly failed to consider the claimed invention "as a whole," as required by the statutory language of § 103. Accordingly, it is respectfully submitted that the Examiner has failed to teach or suggest all claim limitations, as required under § 103. Reversal is respectfully requested.

b. The Examiner's Asserted Motivation to Combine References Lacks a Rational Underpinning

The Examiner's asserted basis for combining references is that HMTBA is a nutrient and that organic acids are generally known or assumed to have some antimicrobial properties. The Appellant respectfully asserts that this reason to combine is overbroad, lacks a rational underpinning, and is contrary to the prior art of record as exemplified by Enthoven. If the claimed organic acids are known

to be ineffective against a particular microbe, such as *Salmonella* (See Enthoven), then there is no rational motivation to combine them for the claimed method of use. In the present case, the prior art and the Examiner admitted that there are no specific teachings regarding a number of the claimed organic acids and Enthoven teaches away regarding HMTBA. Importantly, every organic acid combination recited in the claims requires HMTBA. Next, one of skill in the art would appreciate that there is a high degree of unpredictability regarding the chemical arts, and that the addition or subtraction of an individual ingredient may substantially alter the properties of the overall organic acid blend. Reversal is respectfully requested.

c. The Examiner's Asserted Combination Fails to Provide a Reasonable Expectation of Success

In view of the foregoing comments and arguments, which are hereby incorporated and reasserted, the Appellant respectfully asserts that the Office's asserted basis for rejection provides no reasonable expectation of success in arriving at the currently claimed invention. In particular, the Office fails to identify which combinations of organic acids, if any, are effective at killing *Salmonella* in food. The identification of separate ingredients in different prior art references, without regard to whether they are the critical ingredient or effective at killing *Salmonella* on their own, is an insufficient basis for rejection under § 103.

There may be an infinite number of food additives that could potentially be used to modify the microbial characteristics of a food composition. There is no indication in the prior art, however, which additives or combination of additives are critical at inhibiting or killing *Salmonella* in food. This is particularly true since the Ivey reference[16] (col. 6, lines 8-10), has shown that some microorganisms may grow and thrive in acidic environments, including those containing Alimet and propionic acid. Reversal is respectfully requested.

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[16] The Ivey reference was cited by the Office in the Final Office Action mailed December 11, 2007, as well as the Non-Final Office Action mailed March 27, 2007.

d. Blake *et al.* ("Blake") Teaches Away; Blake Describes Methionine Analogues Not Useful as Animal Food Supplements

The Final Action's reliance on the Blake patent is also misplaced. The passages cited and relied on by the Office (*i.e.*, Blake *et al.*, at col. 1, lines 39-41) does not actually refer to 2-hydroxy-4-(methylthio)butanoic acid but to new chemical variants with substantially different molecular structure and function.

> "Other methionine analogues **differ considerably from the natural methionine in molecular structure** and because of the unnatural configuration are **not useful as animal feed supplements**. Many of these are absorbed by the plant and animal structures and **have toxic effects** due to the inability of the organism to assimilate the analogue . . . Thus many of the new compounds are useful as fungicides, bactericides, virus control agents [etc.]"[17]

Even though the new compounds are deadly to microorganisms, the Blake patent **teaches away** from use of these variants in animal food and water by reciting they are "**not useful as animal feed supplements**" and "**have toxic effects**." (Emphasis Added). Even if these toxic chemicals could theoretically be regulated as applied to the surface of plants or animals to remove certain microorganisms, the Blake patent **provides no teachings for how these variant chemicals could be ingested or combined with food supplements**. Therefore, these toxic analogues are considerably different from the previously known animal feed additives and methionine derivatives. Blake fails to provide the necessary teachings as relied upon by the Final Action, and teaches away since the new compounds are not useful for animal feed supplements. More importantly, Blake does not disclose or suggest the use of the presently claimed HMTBA, a methionine derivative.

[17] See, *e.g.*, Blake *et al.* at col. 1, lines 31-42. (Emphasis Added).

e. **Bland *et al.* ("Bland") Teaches Away; Organic Acids Are Not Effective At Killing Bacteria in Foodstuffs Without Formaldehyde**

The Office relies on Bland to support that the animal feed composition[s] comprise antibacterial agents formic acid, propionic acid, lactic acid.[18] Although the cited animal feed compositions do include these ingredients, Bland *et al.* states that the ingredients are not effective at killing bacteria in animal feedstuffs, including *Salmonella*.

> "**[M]any compounds with known bacteriocidal properties**, such as lactic acid, propionic acid, formic acid, butyric acid, sorbic acid, benzoic acid and combinations of these have been tested. While many of these agents kill bacteria in solution, **they do not kill all the bacteria in animal feedstuffs**. Woolford, M. K., "Microbiological Screening of Food Preservatives, Cold Sterilants and Specific Antimicrobial Agents as Potential Silage Additives", J. Sci. Ed. Agric. 1975, 26, 229-237. **To be effective against Salmonella, a bacteriocidal treatment must kill essentially all of the bacteria**. Methods that kill 95% or even 99% are largely ineffective because the residual bacteria can multiply rapidly and recontaminate the feedstuff, and eventually the entire processing facility."[19]

"A prior art reference must be considered in its entirety, *i.e.*, as a whole, including portions that would lead away from the claimed invention."[20] Taken in context, **Bland actually teaches that the required key ingredient for a bactericidal composition is formaldehyde**. (Emphasis Added). As such, it is respectfully asserted that the Office cannot merely show that certain components are

[18] See, *e.g.*, Final Action at page 4, lines 19-21.
[19] See, *e.g.*, Bland *et al.* at col. 2, lines 20-34. (Emphasis Added).
[20] See, e.g., MPEP § 2141.02; *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540 (Fed. Cir. 1983).

effective in solution, if they are not effective at killing *Salmonella* in food, as required by claim 75.

> "[S]uch **treatments fail to eliminate the Salmonella effectively when too little formaldehyde is used** or when the solution is not sprayed uniformly onto the feedstuff, thereby allowing some small number of bacteria to survive and multiply."[21]

Because one of skill in the art would view all embodiments of the Bland patent to expressly or implicitly require formaldehyde in order to successfully inhibit or kill microbes in food compositions, the Bland reference may be said to teach away. The currently claimed invention does not recite or require any formaldehyde. Consequently, Bland provides no expectation of success for using organic acids without the addition of large amounts of formaldehyde.

f. The Buttin Reference is Cited Out of Context

The Buttin reference indicates that there is greater weight gain in pig diets having DL-HMB instead of DL-methionine as a protein source. There is no statement regarding DL-HMB having an antimicrobial effect on any specific microbe or microbes generally. The Buttin references states that, "The recent ban on antibiotic growth promotants has dramatically reinforced interest in the benefits of diet acidification." However, this fails to indicate that HMBA, or any other acid that contributes to acidification, is effective against any particular microbe or *Salmonella* in particular. The Appellant respectfully asserts that the Examiner has cited Buttin out of context.

g. *In re Kerkhoven* Does Not Properly Apply

Due to the contradictory teachings of the prior art as indicated above, the Office's reliance on *In re Kerkhoven*, 626 F.2d 848 (CCPA 1980) in the Final

[21] See, *e.g.*, Bland *et al.* at col. 2, lines 39-43. (Emphasis Added).

Action is misplaced. To reiterate, none of the currently cited reference discloses or suggests the use of HMTBA as an antimicrobial effective against *Salmonella* in food, as presently claimed. Every currently pending claim recites HMTBA as part of a method of killing or inhibiting *Salmonella* in food.

In re Kerkhoven was cited on the belief that the cited references show antimicrobial agents that are useful for the same purpose. However, close evaluation of the cited prior art has revealed that these teachings do not exist or, alternatively, that the individual components are ineffective or insufficient for the purposes of the currently claimed invention – killing or inhibiting *Salmonella* in food. In a number of instances, it has been shown that the prior art actually teaches away from the claimed methods. For example, Enthoven states that HMBA is ineffective at inhibiting *Salmonella*. While Blake describes variants of methionine with toxic effects, these particular chemicals are recited as unacceptable for animal food. Finally, Bland teaches away by reciting that several organic acids are bacteriocidal in solution, but are insufficient at killing bacteria in feedstuffs without large amounts of formaldehyde. As such, the currently claimed invention is not taught or suggested by the prior art, and *In re Kerkhoven* does not properly apply.

h. Previously Submitted Evidence Supports a Finding of Non-Obviousness

The Declaration of Dr. Knight under 37 C.F.R. §1.132 shows that the individual organic acids are inadequate for the limitations of the claimed invention. The following passage from the Declaration states that the methods of the claimed invention also demonstrate unexpected results.

> "… [w]e have research data, that in my opinion, **<u>demonstrates surprising and unexpected results for organic acid formulations falling within the scope of the '434 patent claims</u>**. As an example,

> attached to this Declaration is a graph (identified as figure 7) that depicts a synergistic effect . . ."[22]

Also, every organic acid recited in claim 75, when tried alone, was not effective at killing *Salmonella* in food. As such, the Declaration is evidence that further supports allowance of the presently pending claims.

In addition to the Declaration of Dr. Knight, the Appellant also previously submitted the Warnecke *et al.* review article as part of the response to the non-final Office Action mailed March 27, 2007.[23] The Warnecke *et al.* review article cites to work done pre-filing to which a skilled artisan would appreciate as indicating unpredictability in the microbial arts. Thus, the general state of knowledge in the microbial arts at the time of filing supports the notion that a random selection of organic acids would be unpredictable for the purpose of the currently claimed invention. The Warnecke *et al.* review article exemplifies this unpredictability, and reveals that many microorganisms may live and thrive in acidic environments.[24] Individual organic acids uniquely, and at times unpredictably, impact microbe cell growth, regulatory pathway, turgor pressure, and cell landscape.[25] Every organic acid may potentially cause a unique

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[22] 37 C.F.R 1.132 Declaration of Dr. Christopher Knight, at paragraph 4, a copy of which was submitted with the response to the Office Action dated March 27, 2007. (Emphasis Added).
[23] A copy of the Warnecke *et al.* review article was submitted to the USPTO on September 26, 2007, as part of the response to the non-final Office Action mailed March 27, 2007.
[24] Warnecke, T., and Gill, R., Microbial Cell Factories (2005) 4:25, a copy of which was submitted with the response to the Office Action dated March 27, 2007.
[25] *Id.* For example, see the third page, column two of the article, which states:
Organic acid anions affect cell growth in a variety of manners. Increased anion concentration has been shown to lead to an increased transport of potassium ions into the cell, which increases turgor pressure [47,48]. To maintain a constant turgor pressure and cell volume, glutamate is transported out of the cell [48]. This transport activity concomitantly disrupts the osmolarity of the cytoplasm, which in turn lowers the cell's growth potential and viability. In addition to this general anion effect, **there are also effects specific to each organic acid.** It has been proposed that enzymes involved in protein synthesis are sensitive to a combination of two unrelated mechanisms, including the acidification of pHi and the formation of an anionic pool [35]. Although this finding implies that the **organic inhibition due to the anion pool could be acid specific**, the details describing this dual inhibition mechanism remain unclear. Kirkpatrick et al. reported proteins exhibiting increased expression in response to extracellular acetate [33]. Among these are the OppA transporter, RpoS regulon, several amino acid uptake proteins, DNA binding proteins, and extreme-acid preiplasmic chaperones. Interestingly, when formate was introduced in place of acetate the expression of the previously mentioned proteins was repressed,

response by an individual microorganism. Additionally, the degree of bioavailability (*i.e.*, ability to reach the target microbe) varies for different organic acids, and different microbes are resistant to different pH ranges. With this degree of unpredictability, a skilled artisan empowered with the cited prior art and the general knowledge of the microbial arts would not have a reasonable expectation of success in combining references as indicated by the Final Action.

"Evidence rebutting a *prima face* case of obviousness can include: 'evidence of unexpected results,' [and] evidence 'that the prior art teaches away from the claimed invention in any material respect' . . . When a patent Appellant puts forth rebuttal evidence, the Board must consider that evidence."[26] In the present case, the Appellant has previously submitted substantial evidence of unexpected results to rebut a finding of obviousness. While synergism is not a requirement of non-obviousness,[27] it has been shown that synergism and unexpected results exist in the present case. The combination of the claimed invention is greater than the additive effect of what would be expected from the sum of the individual components. In fact, as exemplified in Figure 7, none of the recited organic acids in claim 75 were effecting at killing *Salmonella* in food. When synergism is present, particularly in a chemical case, it is indicative of non-obviousness.[28]

The data of record shows that the combination of the claimed invention kills a substantially greater number of microbial colonies, as compared to the organic acids tested. For example, in Figure 7 that accompanied the Declaration of Dr. Knight, Blend OA 4 and Blend OA 6 were shown to have approximately a 10-fold improvement compared to any of the single organic acid compositions tested at equivalent volumes. Blends OA 4 and OA 6 are embodied by the

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indicating that the response was anion specific. This finding introduces new challenges in addressing organic acid tolerance. Specifically, it highlights the need to engineer both pH and as well as specific anion tolerance into host organisms. (Emphasis added).

[26] See, *e.g.*, *In re Sullivan*, 498 F.3d 1345, 1351 (Fed. Cir. 2007) (internal citations omitted).

[27] *Gardner v. TEC Sys. Inc.*, 725 F.2d 1338, 1349 (Fed. Cir. 1984) (en banc).

[28] *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1540 (Fed. Cir. 1983).

currently claimed invention. By way of example, claims 127 and 128 specifically recite the composition of Blend OA 6.

Among other considerations, the previously cited Ivey reference[29] provides evidence that propionic acid in combination Alimet does not reliably inhibit or kill microbes in food or water. In the non-final Office Action mailed March 27, 2007, the Examiner originally asserted at page 5, lines 4-7, that "Ivey's method inherently inhibits or kills microbes in a subject such as poultry feed, since the method steps are same as the instant method steps, mixing the same compound in the same effective amount to the same subject will cause the same effect, whether or not that effect is specifically disclosed by the prior art." (Emphasis Added). However, **it was later identified by the Appellant that Ivey's methods are not inherently antimicrobial, since the Ivey composition is used to deliver a probiotic or microbial to the animal**. See, *e.g.*, Appeal Brief Filed September 10, 2008, at pages 8-9 (below excerpts taken from previously filed Appeal Brief)."

> "The high moisture solid of the present invention, therefore, may be used as a **vehicle to administer direct-fed microbials to poultry and other animals.** When used for this purpose, the high moisture solid **should contain sufficient colony forming units of the yeast or bacterium to be of benefit to the animal.**"[21]
>
> "The present invention is also directed to a composition and process for **inoculating poultry and other animals with living cells such as yeast or bacteria**"[22]
>
> [21] See, *e.g.*, Ivey *et al.* at col. 6, lines 8-19. (Emphasis Added).
> [22] See, *e.g.*, *id.* at col. 2, lines 65-67. (Emphasis Added).

[29] The Ivey reference was cited by the Office in the Final Office Action mailed December 11, 2007, as well as the Non-Final Office Action mailed March 27, 2007.

Similarly, the previously cited Bland reference states that "... **many compounds with known bacteriocidal properties, such as ... propionic acid** ...and combinations of these have been tested. **While many of these agents kill bacteria in solution, they do not kill all the bacteria in animal feedstuffs.**"[30] Therefore, the general state of the art supports the non-obviousness evidence submitted by the Appellant. Finally, previously submitted data shown in Figure 7, including a comparison between propionic acid and HMTBA, indicated that propionic acid alone is ineffective for the purposes of the claimed invention.[31] Again, because the provided evidence and experimental results shows that each individual organic acid was not effective at killing or inhibiting *Salmonella* in food, the currently claimed invention combination of elements is both surprising and unexpected over the prior art. As such, the evidence of record plainly shows that the currently claimed invention is, as a whole, non-obvious. Reversal is respectfully requested.

2. The Group II Claims Under Rejection (Claims 86 and 87)

Claim 86 is representative of the Group II claims under rejection. Claim 86 is directed to a method of killing *Salmonella* in food. The method comprises treating the food or water with an antimicrobial composition, and feeding to **a ruminant animal**. (Emphasis Added). The antimicrobial composition comprises 2-hydroxy-4-(methylthio)butanoic acid and at least two organic acids chosen from butyric acid, lactic acid, and propionic acid. The organic acid composition inhibits

[30] See, *e.g.*, Bland *et al.* at col. 2, lines 20-34. (Emphasis Added).
[31] The comparison between propionic acid and HMTBA for Salmonella was previously submitted by the Appellants and entered into the record pursuant to 37 C.F.R. §1.116(e). The Advisory Action mailed May 14, 2008 indicated that the request for reconsideration had been considered and made of record, even though the specifically requested claim amendments were not permitted. Furthermore, the Appellants had good and sufficient reasons why the affidavit was necessary and was not earlier presented, since the Examiner had specifically stated that "HMTBA is not convincing because no data is provided for the propionic acid alone for comparison." See, *e.g.*, Final Action at page 16, lines 22-23. As such, the recited evidence was properly entered into the record.

or kills more *Salmonella* in the food compared to when the food is treated with any single organic acid that forms the organic acid composition

The arguments asserted above are hereby incorporated reasserted with respect to claims 86 and 87. In particular, the cited references have been previously shown above to teach away from the currently claimed invention, provide no motivation to combine, and give no expectation of success regarding inhibiting or killing *Salmonella* in food. More specifically, the cited art combination provides no motivation to combine and no expectation of success with particular regard to **ruminant animals.** (Emphasis Added). Reversal is respectfully requested.

3. The Group III Claims Under Rejection (Claims 115-117)

Claim 116 is representative of the Group III claims under rejection. Claim 115 is directed to a method of killing *Salmonella* in food as recited in Claim 75 (as discussed above), but additionally requires the composition having a pH of about 4 to about 5.

The arguments asserted above) are hereby incorporated and reasserted with respect to claims 115-117. In particular, the cited references been previously shown to teach away from the currently claimed invention, provide no motivation to combine, and give no expectation of success regarding inhibiting or killing microbes in food or water. The cited art combination provides no motivation to combine and no expectation of success regarding **composition having a pH of about 4 to about 5** for a method of inhibiting or killing *Salmonella* in food. (Emphasis Added). Reversal is respectfully requested.

4. The Group IV Claims Under Rejection (Claim 121-122, 124-125, 127-128, and 130-131)

Claim 122 is representative of the Group IV claims under rejection. Claim 122 is directed to a method of killing *Salmonella* in food (as discussed above), but additionally recites that the content of 2-hydroxy-4-(methylthio)butanoic acid is from about 20% to about 40% of the sum of the 2-hydroxy-4-(methylthio)butanoic acid, butyric acid, and lactic acid content; the content of the butyric acid is from about 10% to about 30% of said sum; and the content of the lactic acid is from about 10% to about 30% of said sum.

The arguments asserted above are hereby incorporated and reasserted with respect to claims 121-122, 124-125, 127-128, and 130-131. In particular, the cited references have been previously shown to teach away from the currently claimed invention, provide no motivation to combine, and give no expectation of success regarding inhibiting or killing *Salmonella* in food. Even more specifically, the cited art combination provides no motivation to combine and no expectation of success regarding **specific percentages** of 2-hydroxy-4-(methylthio)butanoic acid, formic acid, and propionic acid **as claimed by the Group IV claims.** (Emphasis Added). The teachings away as identified above and the unpredictability in the microbial arts indicate that the claimed percentages would not have been within the skill in the art. As such, the Office's reliance on *In re Bosch*, 205 USPQ 215 (CCPA 1980) for the selection of optimal parameters[32] is not supported by either the cited art or the general state of the technology. Reversal is respectfully requested.

G. The Rejection of Claims 88-89 under 35 U.S.C. 103(a) over Dunn et al., Blake et al., Buttin, Bland et al., and Pinski et al. is Improper

Claims 88-89 are directed to methods of killing *Salmonella* in food fed to an aquaculture animal and belong to the Group I claims. Claim 75 is representative of the Group I claims. The arguments asserted above are hereby incorporated reasserted with respect to claims 88-89.

[32] See, *e.g.*, Final Action at page 7, lines 7-9. (Emphasis Added).

The cited references have been previously shown to teach away from the currently claimed invention, provide no motivation to combine, and give no expectation of success regarding inhibiting *Salmonella* in food. The Office has cited Pinksi because it generally relates to aquaculture and is said to disclose antimicrobial agents "selected from propionic acid, salt of propionic acid, citric acid, or a salt thereof."[33]

The teachings of Pinski are limited to oil-coated, encapsulated, moistured aquaculture feed having a particle size of less than about 1000 micrometers. Pinksi provides no teachings for foods that are not oil-coated and encapsulated. Pinski also teaches away from the claimed invention by packaging foodstuff with bacteria that do not appear to be adversely affected, inhibited, or killed by the so-called antimicrobials. [34]

> "In one aspect, powdered feed, endo-probiotic bacteria and/or ecto-probiotic bacteria, water and oil are mixed to provide a feed which not only can enhance the value of the feed for certain species of aquatic life, such as shrimp, but the release of such bacteria can help maintain a clean water environment . . . **Endo-probiotic bacteria which may be used in the product of the invention include dried B. licheniformis and B. subtilis strains commercially available** . . ."[35]

It has also been shown by Bland that the specific organic acids listed by Pinski[36] are not effective at killing microbes in animal feedstuffs without large amounts of formaldehyde. This teaching away by Bland contradicts any supposed motivation to combine the references cited in this rejection, and supports a finding of non-obviousness. Reversal is respectfully requested.

[33] See, e.g., Final Action at page 8, lines 1-2.
[34] See, *e.g.*, Pinski *et al.* at published paragraph [0015].
[35] See, *e.g.*, *id.* (Emphasis Added)
[36] See, *e.g.*, *id.* at published paragraph [0010].

H. The Rejection of Claims 94-95 under 35 U.S.C. 103(a) Dunn et al., Blake et al., Buttin, Bland et al., and Friedman et al. ("Friedman") is Improper

Claims 94-95 are directed to a method of killing or inhibiting *Salmonella* in food that is fed to a companion animal, and belong to the Group I claims. Claim 75 is representative of the Group I claims. The arguments asserted above are hereby incorporated and reasserted with respect to claims 94-95. The Office cites Friedman because it is alleged that it teaches "pet food for feeding pets such as dog food contains antibacterial agents."[37]

The cited references have been previously shown to teach away from the currently claimed invention, provide no motivation to combine, and give no expectation of success regarding inhibiting or killing microbes in food. **Notably, the cited prior art combination fails to teach a composition that would have a reasonable expectation of success at killing *Salmonella* in food.**

Friedman does not teach, disclose, or suggest HMBA as claimed by the Appellant. It has also been shown by Bland that the organic acids disclosed by Friedman[38] are not effective at killing microbes in animal feedstuffs without large amounts of formaldehyde. This teaching away by Bland contradicts any supposed motivation to combine or expectation of success, and supports a finding of non-obviousness. Reversal is respectfully requested.

I. The Rejection of Claims 114, 123, 126, 129, and 132 under 35 U.S.C. 103(a) over Dunn, Blake, Buttin, Bland, and Rolow *et al.* ("Rolow") is Improper

1. The Group III Claims Under Rejection (Claim 114)

Claim 114 is representative of the Group III claims under rejection. The method of claim 114 is dependent on claim 75, but further comprises **an acidulant selected from the group consisting of phosphoric acid, sulfuric**

[37] See, *e.g.*, Final Action at page 8, lines 20-21.
[38] See, *e.g.*, Friedman et al. at col. 3, lines 64-67, col. 4, lines 1-16.

acid, phosphorous acid, hydrochloric acid, hydrobromic acid, and nitric acid. (Emphasis Added).

The arguments asserted above are hereby incorporated and reasserted with respect to claim 114. The Office cites the Rolow reference as allegedly disclosing a liquid preservation composition to extend the shelf life of tortillas made from corn. In a preferred embodiment, the preservation composition of Rolow is said to comprise "phosphoric acid, propionic acid, and benzoic acid."[39]

The cited references have been previously shown to teach away from the currently claimed invention, provide no motivation to combine, and give no expectation of success regarding inhibiting or killing microbes in food or water. **Notably, the cited prior art combination fails to teach a composition that would have a reasonable expectation of success at killing *Salmonella* in food.**

More specifically, Rolow is limited to tortillas and products made from tortilla flour.[40, 41] Rolow indicates that a number of known antimicrobial preservatives, including those claimed by Appellant, are unacceptable for individual use in tortillas because they adversely affect taste and odor.[42] **Rolow specifically identifies fumaric acid and benzoic acid to be unsatisfactory as individual antimicrobial agents in tortillas, giving an off flavor and being ineffective at controlling growth of high level organisms**.[43, 44] (Emphasis

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[39] See, *e.g.*, Final Action at page 9, lines 18-21.
[40] "[I]t can be seen that the combination of benzoic acid with propionic acid and phosphoric acid, in the proportions specified, is an effective preservative for products made from tortilla flour." (See, e.g., Rolow *et al.* at col.7, lines 47-51).
[41] "This invention relates generally to methods and chemicals for extending the shelf life of corn tortillas or wheat tortillas, and specifically the preservation of corn tortillas or wheat tortillas . . ." (See, *e.g.*, *id.* at col. 1, lines 12-15).
[42] "Various antimicrobial preservatives have been proposed, however they have limitations of increasing the cost of producing tortilla and/or adversely affecting the taste and odor." (See, *e.g.*, *id.* at col. 1, lines 35-38).
[43] "[A]cidulants such as fumaric acid or citric acid, are used to reduce pH levels. A major drawback resulting from this type of preservative mixture is the lingering after-taste of the acidulant. These preservative mixtures have successfully increased the shelf life of tortillas . . . However, the taste of the tortillas containing these preservatives has not been satisfactory. Also the supply of some of these preservatives have been limited, making them difficult to or expensive to obtain." (See, *e.g.*, *id.* at col. 2, lines 5-13).

Added). Fumaric acid and benzoic acid are two of the organic acids specifically recited in the Group III claims. The teachings away by Rolow may not be disregarded, since two of the "primary indications of spoilage in tortillas is an off odor or taste . . ."[45] Taken in context, it is apparent that only the specific combination of acids described by Rolow actually yields a "surprisingly . . . fresh taste with a slight sweetness at the finish" for tortillas.[46, 47] One of skill in the art would therefore view Rolow as being **<u>limited to tortilla products and ineffective at controlling growth of high level organisms</u>**.[48] At a minimum, it is entirely unclear whether the Rolow tortilla preservative composition would have any inhibitory effect on *Salmonella*. Reversal is respectfully requested.

2. The Group IV Claims Under Rejection (Claims 123, 126, 129, and 132)

Claim 123 is representative of the Group IV claims under rejection. Claim 120 is directed to a method of inhibiting or killing *Salmonella* in food. The method comprises treating the food or water with an antimicrobial composition. The antimicrobial composition comprises at least two organic acids selected from the group consisting of formic acid, butyric acid, fumaric acid, lactic acid, benzoic acid, and propionic acid; and a third organic acid that is a compound of formula (I). More specifically, **<u>claim 123 requires the content of the phosphoric acid is from about 20% to about 40% of said sum.</u>**. (Emphasis Added).

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[44] "Benzoic acid is a well-known food preservative . . . generally used only in very acidic foods such as pickles, soft drinks and dressings. . . Benzoic acid is also known to impart an off flavor. Because of the narrow pH range in which it has generally been effective and because of its off-flavor, it is being replaced by other preservatives. Benzoic acid has not been effective to control the growth of high-levels of microorganisms. Because tortillas generally have a pH level above the optimum effective antimicrobial range of benzoic acid, benzoic acid has not been commonly used as a tortilla preservative." (See, *e.g.*, *id.* at col. 3, lines 12-27) (internal citations omitted).

[45] See, *e.g.*, *id.* at col. 1, lines 35-38.

[46] See, *e.g.*, *id.* at col. 4, lines 16-19.

[47] See, *e.g.*, *id.* at col. 4, lines 43-46.

[48] *Id.*

The arguments asserted above in are hereby incorporated reasserted with respect to claims 123, 126, 129, and 132. In particular, the cited references have been previously shown to teach away from the currently claimed invention, provide no motivation to combine, and give no expectation of success regarding inhibiting or killing microbes in food or water. Even more specifically, the cited art combination provides no motivation to combine and no expectation of success regarding **<u>specific the percentages as recited by claim 123, 126, 129, and 132.</u>** (Emphasis Added). Reversal is respectfully requested.

J. Conclusion

For the foregoing reasons, the Appellant respectfully submits that the currently pending claims are patentable over the prior art, and request that the rejection of these claims as being unpatentable under 35 U.S.C. § 103 (a) be reversed. The Commissioner is hereby authorized to change any and all fees that may be required or credit any overpayment to Deposit Account No. 50-1662.

Polsinelli Shughart PC

Respectfully submitted,

Date: September 23, 2010

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<u>Claims Appendix to Appeal Brief Under Rule 47.37(c)(1)(viii)</u>

Claims1-74 (canceled).

Claim 75 (previously presented). A method of inhibiting or killing microbes comprising *Salmonella* in food, the method comprising treating the food with an organic acid composition comprising at least three organic acids, the organic acid composition comprising 2-hydroxy-4-(methylthio)butanoic acid and at least two organic acids chosen from butyric acid, lactic acid, and propionic acid, wherein the organic acid composition inhibits or kills more *Salmonella* in the food compared to when the food is treated with any single organic acid that forms the organic acid composition.

Claim 76 (canceled).

Claim 77 (previously presented). The method of claim 75 wherein said food is selected from the group consisting of human food, livestock food, pet food, or aquaculture food.

Claim 78 (previously presented). The method of claim 77 wherein said composition is mixed with the food as it is formulated.

Claim 79 (previously presented). The method of claim 78 wherein said composition is applied to a pre-mixed or pre-pelleted feed.

Claim 80 (previously presented). The method of claim 79 wherein said composition, subsequent to treating said food, is uniformly dispersed throughout said food.

Claim 81 (previously presented). The method of claim 75 wherein said food comprises a meat or bone meal.

Claim 82 (previously presented). The method of claim 75 wherein said food is dry food.

Claim 83 (previously presented). The method of claim 75 wherein said food is liquid food.

Claim 84 (previously presented). The method of claim 75 wherein said food is a combination of dry feed and liquid food.

Claim 85 (previously presented). The method of claim 75 wherein said food is fed to an animal.

Claim 86 (previously presented). The method of claim 85 wherein said animal is a ruminant animal.

Claim 87 (previously presented). The method of claim 86 wherein said ruminant animal is selected from the group consisting of dairy cows, lactating dairy cows, dairy calves, beef cattle, sheep, and goats.

Claim 88 (previously presented). The method of claim 85 wherein said animal is an aquaculture.

Claim 89 (previously presented). The method of claim 88 wherein said aquaculture is fish or crustaceans.

Claim 90 (previously presented). The method of claim 85 wherein said animal is livestock.

Claim 91 (previously presented). The method of claim 90 wherein said livestock is swine or horses.

Claim 92 (previously presented). The method of claim 85 wherein said animal is poultry.

Claim 93 (previously presented). The method of claim 92 wherein said poultry is selected from the group consisting of chickens, turkeys, and hatchlings thereof.

Claim 94 (previously presented). The method of claim 85 wherein said animal is a companion animal.

Claim 95 (previously presented). The method of claim 94 wherein said companion animal is a dog or a cat.

Claims 96 to 113 (canceled).

Claim 114 (previously presented). The method of claim 75, further comprising an acidulant selected from the group consisting of phosphoric acid, sulfuric acid, phosphorous acid, hydrochloric acid, hydrobromic acid, and nitric acid.

Claim 115 (previously presented). The method of claim 75, wherein the composition has a pH of less than about 5.

Claim 116 (previously presented). The method of claim 75, wherein the composition has a pH of about 4 to about 5.

Claim 117 (previously presented). The method of claim 75, wherein the composition has a pH of about 4.5.

Claims 118 to 120 (canceled).

Claim 121 (previously presented). The method of claim 75, wherein the organic acid composition comprises 2-hydroxy-4-(methylthio)butanoic acid, butyric acid, and lactic acid.

Claim 122 (previously presented). The method of claim 121, wherein the content of 2-hydroxy-4-(methylthio)butanoic acid is from about 20% to about 40% of the sum of the 2-hydroxy-4-(methylthio)butanoic acid, butyric acid, and lactic acid content; the content of the butyric acid is from about 10% to about 30% of said sum; and the content of the lactic acid is from about 10% to about 30% of said sum.

Claim 123 (previously presented). The method of claim 122, further comprising phosphoric acid, wherein the content of the phosphoric acid is from about 20% to about 40% of said sum.

Claim 124 (previously presented). The method of claim 135, wherein the organic acid composition comprises 2-hydroxy-4-(methylthio)butanoic acid, butyric acid, formic acid, and lactic acid.

Claim 125 (previously presented). The method of claim 124, wherein the content of 2-hydroxy-4-(methylthio)butanoic acid is from about 10% to about 30% of the sum of the 2-hydroxy-4-(methylthio)butanoic acid, butyric acid, formic acid, and lactic acid content; the content of the butyric acid is from about 2% to about 22% of said sum; the content of the formic acid is from about 20% to about 40% of said sum; and the content of the lactic acid is from about 8% to about 28% of said sum.

Claim 126 (previously presented). The method of claim 125, further comprising phosphoric acid, wherein the content of the phosphoric acid is from about 10% to about 30% of said sum.

Claim 127 (previously presented). The method of claim 75, wherein the organic acid composition comprises 2-hydroxy-4-(methylthio)butanoic acid, butyric acid, lactic acid, and propionic acid.

Claim 128 (previously presented). The method of claim 127, wherein the content of 2-hydroxy-4-(methylthio)butanoic acid is from about 10% to about 30% of the sum of the 2-hydroxy-4-(methylthio)butanoic acid, butyric acid, lactic acid, and propionic acid content; the content of the butyric acid is from about 2% to about 22% of said sum; the content of the lactic acid is from about 8% to about 28% of said sum; and the content of the propionic acid is from about 20% to about 40% of said sum.

Claim 129 (previously presented). The method of claim 128, further comprising phosphoric acid, wherein the content of the phosphoric acid is from about 10% to about 30% of said sum.

Claim 130 (previously presented). The method of claim 135, wherein the organic acid composition comprises 2-hydroxy-4-(methylthio)butanoic acid; butyric acid, formic acid, and propionic acid.

Claim 131 (previously presented). The method of claim 130, wherein the content of 2-hydroxy-4-(methylthio)butanoic acid is from about 1% to about 20% of the sum of the 2-hydroxy-4-(methylthio)butanoic acid, butyric acid, formic acid, and propionic acid content; the content of the butyric acid is from about 1% to about 15% of said sum; the content of the formic acid is from about 65% to about 85% of said sum; and the content of the propionic acid is from about 1% to about 15% of said sum.

Claim 132 (previously presented). The method of claim 131, further comprising phosphoric acid, wherein the content of the phosphoric acid is from about 1% to about 15% of said sum.

Claim 133 (canceled).

Claim 134 (previously presented). The method of claim 75, wherein the organic acid composition further comprises at least one organic acid chosen from formic acid, fumaric acid, and benzoic acid acetic acid, malic acid, tartaric acid, mandelic acid, citric acid, sorbic acid, boric acid, succinic acid, adipic acid, glycolic acid, and glutaric acid.

Claim 135 (previously presented). The method of claim 75, wherein the organic acid composition further comprises at least one organic acid chosen from formic acid, fumaric acid, and benzoic acid.

Claim 136 (previously presented). The method of claim 134, further comprising an acidulant selected from the group consisting of phosphoric acid, sulfuric acid, phosphorous acid, hydrochloric acid, hydrobromic acid, and nitric acid.

Claim 137 (previously presented). The method of claim 135, further comprising an acidulant selected from the group consisting of phosphoric acid, sulfuric acid, phosphorous acid, hydrochloric acid, hydrobromic acid, and nitric acid.

Evidence Appendix to Appeal Brief Under Rule 47.37(c)(1)(ix)

A copy of Dr. Knight's Declaration under 37 C.F.R. 1.132 was initially submitted to the USPTO on September 26, 2007, as part of the response to the non-final Office Action mailed March 27, 2007. The response was entered by the Examiner as indicated by the Final Action mailed December 11, 2007. The Declaration was again filed and entered into the record on May 26, 2009. Dr. Knight's previously submitted declaration also included a copy of his curriculum vitae, demonstrating his knowledge and expertise in the technical field. A copy of Dr. Knight's Declaration, as previously submitted, is hereby attached as evidence to the Appeal Brief.

A copy of the Warnecke *et al.* review article under 37 C.F.R. 1.132 was submitted to the USPTO on September 26, 2007, as part of the response to the non-final Office Action mailed March 27, 2007. The response was entered by the Examiner as indicated by the Final Action mailed December 11, 2007. A copy the Warnecke *et al.* review article is hereby attached as evidence to the Appeal Brief.

A copy of the Enthoven and Ivey references, as previously cited by the Office are attached as evidence to the Appeal Brief. The Enthoven reference was cited by the Office in the Non-Final Office Action mailed December 23, 2008. The Ivey reference was cited by the Office in the Final Office Action mailed December 11, 2007. In particular, the Enthoven references states that, "the results show there is **no inhibitory effect of HMB or formic acid on *Lactobacillus* or *Salmonella***." (Note: the terms Alimet®, HMB, HMBA, HMTBA, and 2-hydroxy-4-(methylthio)butanoic acid are used interchangeably.[49]). The Ivey reference, at col. 6, lines 8-10, indicates that, despite having propionic acid, the composition may be used to deliver a probiotic or microbial to an animal.

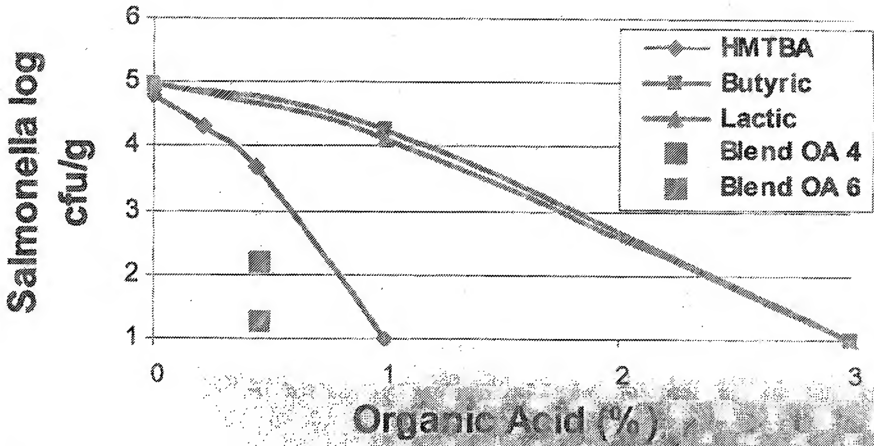
[49] See, *e.g.*, *id.*, at page 36, lines 5-8.

<u>Related Proceedings Appendix to Appeal Brief Under Rule 47.37(c)(1)(x)</u>

There are no related decisions for this appeal.

...HMTBA-containing organic acid blends

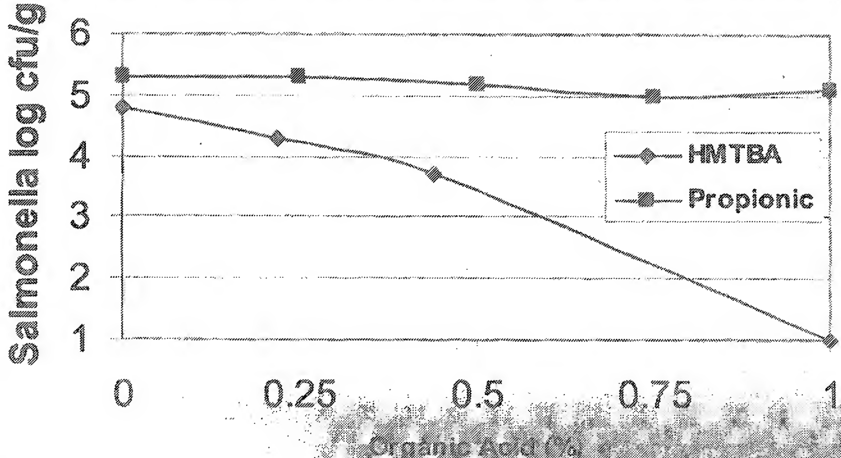
(Activate) against *Salmonella* (in feed for 90min, 37C, pH 4)



NOVUS

Comparison of HMTBA & Propionic on *Salmonella*

in feed for 90min, 37C, pH 4



NOVUS

UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Schasteen et al.	Art Unit	1617
Serial No.:	10/652,745	Examiner:	S. Kantamneni
Filed:	August 29,2003	Conf. No.	1765
For:	ANTIMICROBIAL COMPOSITIONS		

DECLARATION OF CHRISTOPHER D. KNIGHT UNDER 37 C.F.R. § 1.132

I, Christopher D. Knight, declare and state as follows:

1. I have over twenty years of experience in the field of animal health and nutrition. Novus International Inc., a global leader in animal health and nutritional products, currently employs me as Vice-President for Research and Development. My employment by Novus International has been continuous for over sixteen years. Prior to my employment at Novus International Inc., I was employed by Monsanto in their Animal Sciences Division for over five years. My educational background includes a Bachelor of Science degree in Animal science awarded by Cornell University in 1975; a Master of Science degree in Monogastric Nutrition awarded by Purdue University in 1977; and a doctorate degree (i.e., Ph.D.) in Monogastric Nutrition awarded by Purdue University in 1981. I have also published over approximately thirty journal articles or posters at internationally attended meetings, and I am an inventor on three patents. Attached to this Declaration is a copy of my curricula vitae.

2. I have reviewed U.S. Patent Application Publication No. 2004/0175434 ('434 application) entitled "Antimicrobial Compositions." The '434 application has claims directed toward antimicrobial compositions that comprise several organic acid formulations developed at Novus, and presently sold under the trade name ACTIVATE®.

3. Through my position at Novus as Vice-President for Research and Development, I am familiar with and supervised portions of the research and development efforts that resulted in the discovery of several organic acid blends, which are claimed in the '434 application. The focus of this research effort was to improve the cost effectiveness of the formulations, while at the same time improving the antimicrobial activity of the blend of organic acids compared to any individual organic acid comprising the blend. The ACTIVATE® organic acid

formulations (as described in various iterations of the '434 application), in my opinion, meet both of the aforementioned goals.

4. We have research data, that in my opinion, demonstrates surprising and unexpected results for organic acid formulations falling within the scope of the '434 patent claims. As an example, attached to this Declaration is a graph (identified as figure 7) that depicts a synergistic effect for two organic acid formulations of the claimed invention. With reference to the attached graph, data is depicted for the antimicrobial activity of five different organic acid compositions against *Salmonella* in feed. The five organic acid compositions include: (1) 0.45% HMTBA alone (i.e., 2-hydroxy-4-(methylthio)butanoic acid, which is a compound of Formula (I) in the '434 application); (2) 0.45% butyric acid alone; (3) 0.45% lactic acid alone; (4) blend OA 4, which is 0.15% lactic acid, 0.15% propionic acid, and 0.15% HMTBA; and (5) blend OA 6, which is 0.1% lactic acid, 0.1% butyric acid, 0.1% propionic acid, and 0.15% HMTBA. The antimicrobial experiments were conducted in accordance with Novus's standard protocol entitled "Low pH in Feed Test Procedure," a copy of which is attached to this Declaration. As depicted in the graph, the antimicrobial activity of either blend OA 4 or blend OA 6 achieved significantly higher killing of *Salmonella* at lower concentrations than could be achieved with any of the single organic acids alone.

5. I further declare that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Christopher D. Knight — Christopher D. Knight

9/25/2007
Date

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Education

1977- 1981	Ph.D. in Monogastric Nutrition Purdue University, West. Lafayette, IN Department of Animal Science. Graduate Instructorhsip, 1977-1981
1975- 1977	M.S. in Monogastric Nutrition Purdue University, West. Lafayette, IN Department of Animal Science. Graduate Research Assistant
1973- 1975	B.S. Animal Sciences Cornell University, Ithaca, NY
1971- 1973	A.A.S. Science Laboratory Technology State University of New York at Cobleskill

Employment

2001- Present	Department Head, Research & Development Novus International, Inc.
1996- 2001	Director New Business Development Novus International, Inc.
1991- 1995	Manager and Director Nutrition Research Novus International, Inc
1987- 1991	Research Group Leader Monsanto Company Animal Sciences Division Porcine Somatotropin Group
1981- 1986	Research Specialist and Research Group Leader Monsanto Company Alimet Metabolism and Applications Research Group

Key Accomplishments

- Developed foundation data quantifying availability of ALIMET® Feed Supplement as a by-pass methionine source in lactating dairy cattle and methods to predict methionine deficiency using existing nutritional models. These data resolved decades of research work to attempting to commercialize this product application that had failed due to unpredictable field results. The research demonstrated Alimet to be the most cost-effective source of post-ruminal methionine activity available, resulted in a US patent and the development of a $5M/yr business for Novus. As of 2005, a new Ruminant Business Unit of 20 employees and agents and a portfolio of 8 products (including Alimet and MHA) for the dairy industry has been formed.

- Led the development and commercialization of OASIS® Hatchling Supplement, a hydrated nutritional supplement fed to young poultry in transit or to stimulate rapid onset of ad libitum feeding after placement. This patented product developed a new market in the poultry industry based on developmental research at Novus showing the impact of early nutrition on subsequent long term performance and health. Cumulative sales of this niche product have exceeded $4M and resulted in the development of gastrointestinal health as a core research and development competency within Novus.

- Led the technology development, regulatory approval and early commercialization of ADVENT® Coccidiosis Control, an orally applied coccidiosis vaccine based upon technology that permits the in vitro determination of oocyst viability such that a vaccine of consistent potency can be produced and marketed. This represented a new area of technology for Novus and in 2003, a jury of scientists and technology experts from Washington University and St. Louis University awarded the developers of this technology (Dr. Julia Dibner and Dr. Chris Knight) with The St. Louis Technology Award. The Advent Coccisosis Control technology was among eight other winners from approximately 70 nominations in the St. Louis vicinity. In determining winners, the judges considered the scope, economic impact and overall significance of the new technology. Facilitated by the Academy of Science of St. Louis, the judging process also examined the level of sophistication of the entries and the innovation utilized to bring it to fruition. This technology represents a keystone of a business strategy that focuses on gastrointestinal health and drug-free poultry production.

- Established a new cost-efficient method of product development research, to insure Novus' capability to conduct scientifically and commercially relevant research across multiple species without requiring ownership or hands on care and management of research facilities. Initially divested Novus-owned animal research facilities and sought collaborative investment opportunities with scientific professionals in animal agriculture to provide capital for research facilities that would be controlled by the research partner but provide Novus with preferred status for conduct of research. To date we have formed 3 partnerships like this in the US that permits routine product development work in broilers, swine (weaning, grow-finish and lactating sows) and dairy cattle, all in commercial scale production environments. Similar agreements are

under development in Brazil (commercial scale egg layer research) and China (commercial scale swine research including wean, grow-finish and sow nutrition).

- The foundation product for Novus International is ALIMET® Feed Supplement, a source of methionine activity referred to as methionine hydroxyl analog or chemically DL-2-hydroxy-4-(methylthio) butanoic acid. Today this business represents approximately $400M in annual revenue to Novus in a $1B methionine market, however, in 1981 this represented about a $20M business. In the course of my 25 year involvement with this product there has been a heated commercial controversy with respect the relative efficacy of Alimet and the competitive product DL-methonine (DLM). A close colleague (Dr. Julia Dibner) and I have had the responsibility of understanding the absorption, metabolism and utilization of Alimet, how it differs from that of DLM and the impact that the differences have on the commercial value of Alimet relative to DLM. Today based on a variety of independent and collaborative research efforts it is understood that the metabolism of Alimet is very different from DLM, that those differences result in differences in ad libitum feed intake (less than DLM at low supplementation rates, greater than DLM at the maximum response level) resulting in different dose responses for the two methionine sources. A substantial part of the controversy was based on the a priori assumption that the two products must have the same dose response since they both provide methionine. With collaboration with various statistical experts, we have been able to establish that the two products in fact have different dose responses and have described the appropriate statistical methods for comparing two products that exhibit different dose responses (Poult. Sci. 85:947-954). The controversy will continue due to commercial conditions (Alimet is less expensive to manufacture than DLM) , however over the course of 25 years Alimet has continued to grow at a 25% compounded annual growth rate with over a 50% market share in the US. The science applied to this commercial issue has laid the technical foundation that has provided Novus with the technical credibility to expand our product offerings from amino acids into nutritional organic acid blends, organic trace minerals, ingredient preservation and coccidiosis control.

Personal

- Married 1982: Sandra J. Rogers (Purdue Food Science MS 1978).
- Children: Adam (19), Evan (16), Audrey (14)

Community Involvement

- Subdivision Trustee: 1987-1989: Led resolution of road and storm sewer repair dispute
- St. Peter's Episcopal Church:
    - Youth Sponsor: 1984-1988
    - Sunday School Teacher:1992-2006 (Variety of grades and curricula)
    - Vestry: 1989-1993
    - Founding Christian Education Commission & Chair: 1989-1993
    - Confirmation Teacher: 2005-6.
    - Founding and sustaining member of Haven of Grace: Home for unwed mothers
- Hobbies
    - Cooking
    - Gardening
    - Kid's Sports

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2. **Knight, C.D.** and J.J. Dibner (1984) Comparative absorption of 2-hydroxy-4-(methylthio)butanoic acid and L-methionine in the broiler chick. J. Nutr. 114:2179-2186.

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Patents

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3. **Knight, C.D.**, K. Koenig, L. Rode, M. Vandenberg, and M. Vázquez-Añón 2000. Process for optimizing milk production. Patent number 601,753

TITLE: Low pH in Feed Test Procedure

METHOD NO.

MATERIAL: Activate DA™

TEST: Anti-bacterial activity of organic acids measured in feed at low pH

SCOPE: Anti-bacterial activity of organic acids is measured in feed at low pH to simulate the low pH and moisture conditions in the upper digestive tract of animal.

MATERIALS:

1. Finished feed: mash or crumble, swine or poultry
2. Fresh culture of *Salmonella* and *Escherichia coli*
4. Brilliant Green Agar or other selective media for salmonella enumeration
5. MacConkey Agar or other selective media for e. coli enumeration
6. Incubator set at 40C for the assay, and 37C for bacteria enumeration (plating)
7. Pipettes and sterile tips
8. Sterile tubes (50 ml)
9. Hydrochloric acid

SAFETY CONSIDERATIONS:

1. Mouth pipetting is not allowed, automatic pipettes or pipette bulbs must be used.
2. Use appropriate gloves where necessary.
3. Dispose of all hazardous waste properly. Autoclave all wastes containing salmonella or e. coli.

PROCEDURE:

Prepare fresh cultures of salmonella and e. coli:

1. Grow a fresh culture of salmonella or e. coli overnight at 37C in Tryptic Soy Broth (or appropriate media for the particular strain of bacteria)
2. Determine the counts by direct plating
3. Keep the culture at 4C until use. Prepare fresh cultures every 2 weeks.

Determine the amount of HCL needed to bring the feed to pH 4.0

1. Prepare 150mM HCL solution from concentrated HCl (12.1N HCl),
2. Weight out 5g of mash or crumbled feed in 50ml tubes,
3. Add 150mM HCl and DI H2O at different proportions (see the table below) to achieve a total volume of 15 ml,

150mM HCl	7.25 ml	7.50ml	7.75 ml	8 ml	8.25ml
DI H2O	7.75 ml	7.50ml	7.25 ml	7 ml	6.75ml
Total volume	15 ml	15ml	15 ml	15 ml	15 ml

4. Vortex the samples for ~1 min, keep at 40C for ~20min (preferable with mixing) for the pH to equilibrate,

5. Adjust the ratio between HCl and H2O until the pH of the feed is at ~ 4.0 (A range of 3.8 to 4.0 is acceptable).

Set up the following treatments (in 50 ml sterile tubes):

	Treatments	Dose	Reps.	Feed	Inoculant (cfu/g of feed)
1	control		2-3	5 gram	40,000
2	Activate DA	0.3%	2-3	5 gram	40,000
3	Activate DA	0.5%	2-3	5 gram	40,000

1. Weigh out 5g of finished feed in a sterile 50 ml centrifuge tube.
2. Add Activate DA to treatments 2 and 3 (15mg in the 0.3% treatment, and 25mg in the 0.5% treatment).
3. Add HCl and DI H2O to bring the pH to 4.0 (pre-determined for each feed, see the procedures above),
4. Inoculate with Salmonella or E. coli to give a final concentration of 40,000 cfu per ml of sample (40,000 cfu/ml x 15 ml = 600,000 cfu/tube).
5. Incubate the samples for 90 minutes in a 40C incubator (preferably with mixing on an end to end rotator, but not required).
6. At the end of 90 minutes incubation, prepare 1:10 dilution of sample in sterile H2O (1ml sample and 9 ml H2O)
7. Plate the following samples on Brilliant Green agar (*salmonella*) and MacConkey agar (*E. coli*) and incubate plates at 37C overnight.
   100ul of 1:10 dilution from step 6
   100ul of undiluted sample
8. Count colonies the next day, determine cfu/ml sample, and compare with control.

ANALYTICAL TIME:

REFERENCE:
ATTACHMENTS : None

DOCUMENT CONTROL DATES :
Issue & Effective Date:
Prepared/Revised by: Date:
Approved by: Date:

Effect of Alimet and OA on E. Coli in swine diet at pH 4.0 (with pre-equilibrated pH)

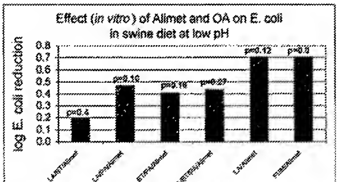
Feed: Ian's diet A, no DLM or alimet
1g feed +2 ml 150mM HCl + 1 ml DH2O, 30mins at 37C before the addition of treatment
E. coli added at 400,000 cfu/g
experiment repeated 2 times, duplicate samples in each run

Treatments:

1	control	
2	0.15% alimet	cont
3	0.15% butyric/0.15% lactic/ 0.15% alimet	Alimet 0.15%
4	0.15% lactic/ 0.15% propionic/ 0.15% alimet	LA/BT/Alimet
5	0.15% butyric/0.15% propionic/ 0.15% alimet	LA/PA/Alimet
6	0.1% lactic/0.1% butyric/0.1% propionic/0.15% alimet	BT/PA/Alimet
7	0.3% lactic/0.15% alimet	LA/BT/PA/Alimet
8	0.3% fumaric/0.15% alimet	LA/Alimet
9	0.3% propionic/0.15% alimet	FUM/Alimet
10	control	PA/Alimet

Treatments	E. coli recovered exp #2		ave	log cfu/g	diff.	exp #1		ave	log cfu/g	diff.	ave (1+2)	ave reduction
1	84000	175000	119500	5.1		632000	436000	534000	5.7		5.3	
2	700	6700	3700	3.6	1.5	400000	234000	317000	5.5	0.2		
3	7000	112000	59500	4.8	0.3	284000	274000	279000	5.4	0.3	5.1	0.2
4	6400	104000	55200	4.7	0.4	85000	82000	83500	4.9	0.8	4.8	0.5
5	57000	35000	* 41000	4.6	0.5	73000	226000	149500	5.2	0.5	4.9	0.4
6	38000	7000	22500	4.4	0.7	228000	250000	239000	5.4	0.3	4.9	0.4
7	21000	2000	11500	4.1	1.0	104000	168000	136000	5.1	0.6	4.6	0.7
8	71000	28000	49500	4.7	0.4	62000	1000	31500	4.5	1.2	4.6	0.7
9												
10	69000	43000	56500	4.7	0.4	420000	316000	368000	5.6			

exp #3		ave	log cfu/g	diff.
6400	3100	4250		
53000	59000	56000	4.7	0.0
100	3500	1800	3.3	1.4
0	700	350	2.5	2.2
500	4300	2400	3.4	1.3
8000	86000	47000	4.7	0.0
3000	117000	60000	4.8	-0.1
6000	1000	3500	3.5	1.2
9000	50000	29500	4.5	0.2
7000	28000	17500	4.2	0.5



P value based on comparison each treatment with control

22

Abstract form

Antibacterial properties of 2-hydroxy-4-(methylthio)butyric acid (HMB, alimet).

P. Enthoven, S. van den Hoven en A. van Dijk. CCL Research, P. O. Box 107, 5460 AC Veghel, The Netherlands.*

Organic acids have many applications in the feed industry e.g. decontamination of raw materials (formic acid) or mold control (propionic acid). Organic acids are also able to modify the gastrointestinal flora which opens perspectives to control Salmonella. To evaluate different products our laboratory developed an in vitro assay in which effects on e.g. Escherichia coli or Salmonella enteritidis can be compared. HMB, a methionine analogue, is also an organic acid.
To investigate the antibacterial properties of HMB we added different amounts of HMB to a buffered broth containing approx. 3.10^5 cfu/ml of a fresh culture of S. enteritidis, E. coli, Lactobacillus plantarum or Campylobacter jejuni. Growth at pH 4.5 and pH 6.75 was determined after 4 or 6 h incubation at 37°C. For comparison the same tests were done with equimolar amounts of formic acid.
The results show that there is no inhibitory effect of HMB or formic acid on Lactobacillus or Salmonella. HMB does show a bactericidal effect at pH 4.5 on Escherichia coli and on Campylobacter at 0.83 g/l. Formic acid has under the same conditions also an effect on Campylobacter but not on E. coli.
Conclusion: In the tested range the antimicrobial effect of HMB is comparable to that of formic acid; given the working mechanism of organic acids it is speculated that these antimicrobial effects are additive.

pH dependant antibacterial effect of formic acid and HMB

CCL, Nl, Jan 2002

in vitro assays with incremental addition of acids in a way to compare weight or molar basis

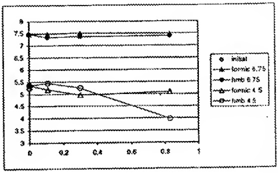
acid in g/l	acid in %	formic mol/l	hmb mol/l
0.108	0.0108	0.002	0.001
0.3	0.03	0.006	0.002
0.83	0.083	0.017	0.006

y = log population, initial and after 4 hours

x = concentration of acid in g/l

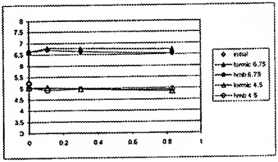
E coli

		t = 0	t = 4h			
	g/l	initial	formic 6.75	hmb 6.75	formic 4.5	hmb 4.5
	0	5.24				
pH 6.75	0		7.47	7.47		
	0.108		7.48	7.33		
	0.3		7.5	7.36		
	0.83		7.49	7.39		
pH 4.5	0				5.36	5.36
	0.108				5.19	5.45
	0.3				4.98	5.25
	0.83				5.08	3.96



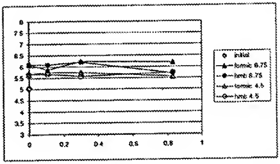
S enteritidis

		t = 0	t = 4h			
	g/l	initial	formic 6.75	hmb 6.75	formic 4.5	hmb 4.5
	0	5.23				
pH 6.75	0		6.62	6.62		
	0.108		6.79	6.71		
	0.3		6.77	6.63		
	0.83		6.72	6.63		
pH 4.5	0				5.03	5.03
	0.108				5.04	4.92
	0.3				4.96	4.96
	0.83				4.86	4.93



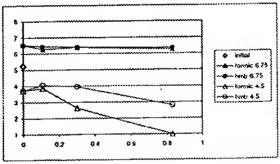
L plantarum

		t = 0	t = 4h			
	g/l	initial	formic 6.75	hmb 6.75	formic 4.5	hmb 4.5
	0	5.04				
pH 6.75	0		6.1	6.1		
	0.108		5.88	6.09		
	0.3		6.23	6.2		
	0.83		6.19	5.7		
pH 4.5	0				5.67	5.67
	0.108				5.75	5.67
	0.3				5.74	5.57
	0.83				5.56	5.74



C jejuni

		t = 0	t = 4h			
	g/l	initial	formic 6.75	hmb 6.75	formic 4.5	hmb 4.5
	0	5.23				
pH 6.75	0		6.54	6.54		
	0.108		6.27	6.44		
	0.3		6.38	6.4		
	0.83		6.25	6.34		
pH 4.5	0				3.7	3.7
	0.108				3.86	4.07
	0.3				2.63	3.95
	0.83				1	2.8



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Review

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Organic acid toxicity, tolerance, and production in *Escherichia coli* biorefining applications

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Abstract

Organic acids are valuable platform chemicals for future biorefining applications. Such applications involve the conversion of low-cost renewable resources to platform sugars, which are then converted to platform chemicals by fermentation and further derivatized to large-volume chemicals through conventional catalytic routes. Organic acids are toxic to many of the microorganisms, such as *Escherichia coli*, proposed to serve as biorefining platform hosts at concentrations well below what is required for economical production. The toxicity is two-fold including not only pH based growth inhibition but also anion-specific effects on metabolism that also affect growth. *E. coli* maintain viability at very low pH through several different tolerance mechanisms including but not limited to the use of decarboxylation reactions that consume protons, ion transporters that remove protons, increased expression of known stress genes, and changing membrane composition. The focus of this mini-review is on organic acid toxicity and associated tolerance mechanisms as well as several examples of successful organic acid production processes for *E. coli*.

Review

Biorefining Platforms

Biorefining promises the development of efficient processes for the conversion of renewable sources of carbon and energy into large volume commodity chemicals. It has been estimated that such bioprocesses already account for 5% of the 1.2 trillion dollar US chemical market [1], with some projecting future values of up to 50% of the total US chemical market generated through biological means. While the attractiveness of such bioprocesses has been recognized for some time [2,3], recent advances in biological engineering and associated sciences [4-15], several biorefining success stories [16-18], and instability in the price and future availability of oil [19], have collectively reinvigorated interest in the large scale production of chemicals through biological routes. Nevertheless, many challenges still remain for the economical bio-production of commodity chemicals. Such challenges encompass the need to not only inexpensively convert biomass into usable sources of carbon and energy but also to engineer microbes to produce relevant chemicals at high titers and productivities while minimizing the generation of byproducts that might foul downstream processes [1,20,21]. One model for addressing the latter of such challenges involves the generation of platform organisms that can be easily engineered and re-engineered to produce a variety of building block chemicals that are amenable to conversions to higher value products via traditional catalytic routes (see Figure 1). Although chemical pretreatment of raw materials impairs viability of platform organisms, this review will focus on product toxicity issues associated with the production of organic acids in

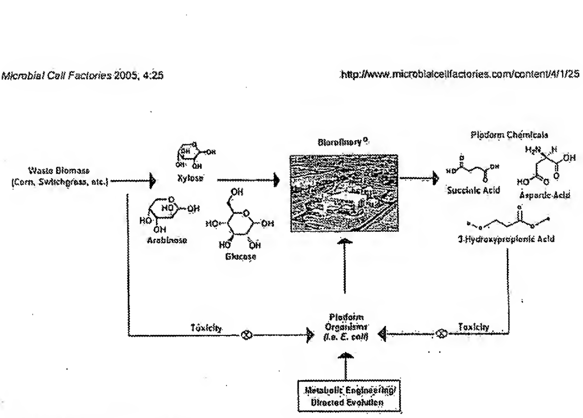

Figure 1
Conceptual model of toxicity in biorefining applications. Sugars are extracted from waste biomass for use as feedstock for platform organisms in a biorefinery. Metabolically engineered microorganisms convert sugars into valuable platform chemicals that are then further derivatized to large-volume chemicals. Product and feedstock toxicity are observed, thus limiting productivity of biorefining applications.

E. coli (for further information on sugar extraction from raw materials see Zaldavar, *et al.* [22] and Knauf, *et al.* [23]).

The US Department of Energy (USDOE) recently released a prioritized list of building block chemicals for future biorefining endeavors. Priority was assigned based on the projected value of the platform chemical and potential derivatives as well as what technological developments were required for the production of the chemical and associated derivatives [21]. The report emphasized the importance of organic acids to the future of biorefining efforts (eight of the top twelve chemicals were organic acids, see Table 1 in additional file 1). The USDOE is not the first to recognize the importance of organic acids. In fact, there is a rich literature describing microbial production of organic acids [17,20,24,25], including several successful commercial bioprocesses [26-28]. Product toxicity is one of the primary challenges in the development of organic acid bioprocesses based on the use of platform host organisms, such as *E. coli*. In particular, while *E. coli* is known to survive very high concentrations of acids (pH = 2) when passing through the mammalian stomach, *E. coli* are surprisingly acid sensitive in exponential phase when cultured planktonically [29,30]. Moreover, undissociated organic acids, which pass freely through the outer and plasma membranes of *E. coli* [31,32], dissociate upon entry into the slightly alkaline cytoplasm releasing protons that lower internal pH (pH_i) and anions that specifically inhibit different aspects of metabolism resulting in impaired growth [33-35]. Titers and productivities of 50–100 g/L and 2–3 g/L·hr are expected for the economical manufacturing of most building block acids by fermentation. The pKa values range from 3–5 for these organic acids, which would result in a pH reduction to around 2.0 for titers of 50 g/L. This highlights a key challenge in the metabolic engineering of organic acid production hosts. That is, high titers result in the addition of protons to the culture, which either result in a decreased pH or the addition of large volumes of base titrant. At low pH, organic acids are undissociated, thus they pass freely through the membrane and inhibit growth. At high pH, the process is less efficient due to base requirements and because export of the organic acid cannot proceed by free diffusion alone (for a more detailed discussion of organic acid export issues see Van Maris *et al.* [36]). What is desired, therefore, is a platform organism that not only produces high levels of organic acid chemicals but also is tolerant to any associated toxicity.

Many microbes are capable of producing platform chemicals by aerobic and anaerobic fermentation processes [22]. L-lactic acid has traditionally been produced by lactic acid bacteria. Although many lactic acid bacteria strains have been studied extensively [37], the ability to produce optically pure L-lactic acid is hampered by the presence of both L and D lactate dehydrogenase genes [38]. Pure L-lactic acid must therefore be produced via another pathway, as the racemic acid product is not useful for downstream conversion into polylactic acid. A number of other microorganisms have been used for industrial fermentation of several of the building block organic acids identified in Table 1. Large scale production of amino acids has been accomplished in *Corynebacteriumglutamicum* [39], succinic acid has been produced by *Actinobacillus succinogenes* [40], and itaconic acid production has been carried out with *Aspergillus terrus* [41]. While successful, the future application of these organisms as platform hosts is limited when compared with *E. coli*. *E. coli* is advantageous as a platform host because it is the most well characterized model organism, it has been used in recombinant processes for over 20 years, there are a wide variety of good genetic tools, and it is sensitive to many antibiotics used in genetic engineering efforts [42]. Moreover, the completion of the *E. coli* genome sequence has already enabled many functional genomics studies and proven useful in metabolic engineering efforts [43]. Finally, *E. coli* grows quickly in minimal media and maintains the ability to metabolize both 5 and 6 carbon sugars, which is a specific advantage over the use of industrially relevant yeast strains [22]. This mini-review will describe the basic mechanisms underlying organic acid toxicity and associated tolerance pathways in *E. coli* followed by a short discussion of several metabolic engineering strategies employed for the production of organic acids in *E. coli*.

Organic Acid Toxicity in E. coli

One of the primary factors contributing to the toxicity of organic acids is their ability to diffuse across *E. coli* cellular membranes when undissociated as opposed to the restricted passage of dissociated protons and anions (see Figure 2) [31,32]. Diffusion of dissociated acids is limited to secondary transport, which is known to involve H+/monocarboxylic acid symporters. However, the detailed mechanism and specificities of the transporters remain unknown [31]. *E. coli* maintain a cytoplasmic pH (pH_i = 7.5) that is most often higher than that of the external media and typically well above the pKa of organic acids [44,45]. As a result, organic acids exist in the dissociated form within the cytoplasm. Thus, diffusing organic acids entering into the cytoplasm will dissociate and disrupt the pHi and anion pool of the cytoplasm. The resulting increase in internal acidity can affect the integrity of purine bases [46] and result in denaturing of essential enzymes inside the cell [35], both of which negatively affect cell viability.

Organic acid anions affect cell growth in a variety of manners. Increased anion concentration has been shown to lead to an increased transport of potassium ions into the cell, which increases turgor pressure [47,48]. To maintain a constant turgor pressure and cell volume, glutamate is transported out of the cell [48]. This transport activity concomitantly disrupts the osmolarity of the cytoplasm, which in turn lowers the cell's growth potential and viability. In addition to this general anion effect, there are also effects specific to each organic acid. It has been proposed that enzymes involved in protein synthesis are sensitive to a combination of two unrelated mechanisms, including the acidification of pHi and the formation of an anionic pool [35]. Although this finding implies that the organic inhibition due to the anion pool could be acid specific, the details describing this dual inhibition mechanism remain unclear. Kirkpatrick et al. reported proteins exhibiting increased expression in response to extracellular acetate [33]. Among these are the OppA transporter, RpoS regulon, several amino acid uptake proteins, DNA binding proteins, and extreme-acid preiplasmic chaperones. Interestingly, when formate was introduced in place of acetate the expression of the previously mentioned proteins was repressed, indicating that the response was anion specific. This finding introduces new challenges in addressing organic acid tolerance. Specifically, it highlights the need to engineer both pH and as well as specific anion tolerance into host organisms.

Finally, production of organic acids might include intermediates that are themselves toxic. For example, 3-hydroxypropionic acid (3HP) is closely related to the antimicrobial compound Reuterin. Reuterin describes the hydroxypropionaldehyde (HPA) system including HPA, HPA dimer, and HPA hydrate. Reuterin is inhibitory to several bacteria, including *E. coli*, at concentrations as low as 0.03–0.05 g/L [49-51]. It is thought that the toxicity could be the result of inhibition of DNA synthesis [52]. It has been postulated that the reactivity of the aldehyde group of HPA causes DNA damage similarly to formaldehyde, which is the aldehyde analog of formic acid [49]. Intermediate toxicity can be managed either by optimization of the production pathway in the host or by engineering tolerance to the intermediate itself.

Organic Acid Tolerance in E. coli

E. coli has a remarkable ability to remain viable under a broad range of pH conditions. This ability is essential for its survival in the mammalian digestive system where pH can vary between pH = 2–8. Several different acid tolerance mechanisms have been identified in *E. coli*. While each mechanism is capable of providing some degree of

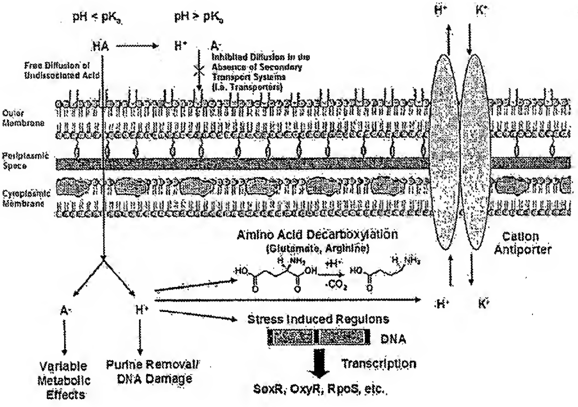

Figure 2
An overview of organic acid toxicity and tolerance mechanisms in *E. coli*. Diffusion of undissociated acid molecules can occur freely in acidic medium but is limited to transport systems at neutral or basic pH. The toxic effects associated with organic acids are the result of both anion specific affects on metabolism as well as increased internal proton concentrations. Affects on internal pH are mitigated by transport of protons out of the membrane, consumption of protons by decarboxylation reactions, and, more generally, induction of stress regulons. Anion specific tolerance mechanisms are not well characterized.

tolerance, they are regulated differently and confer varying levels of tolerance.

Although most acid tolerance systems are activated in stationary phase, acid tolerance as low as pH = 3 has been observed in exponential phase *E. coli* grown under aerobic conditions, which is advantageous from a productivity standpoint [30]. Although the underlying tolerance mechanism is not known, such tolerance can be reliably activated by adapting cells at sublethal pH values between 4.3 and 5.8 [53]. *E. coli* that exhibit growth phase tolerance remain viable at pH values on the same order as stationary phase tolerance, however the percent survival is significantly lower. Lin et al. reported 1% survival of the original culture following acid adaption at pH 4.3 followed by acid challenge at pH 3.3 compared to 0.0001% survival for unadapted cultures. This is compared to stationary-phase cultures, which exhibited up to 50% survival.

Three stationary phase acid resistance systems have been studied in the most detail [29,30]. These systems confer the highest levels of tolerance and are believed to be responsible for stationary phase *E. coli* survival when passing through the mammalian stomach. Acid resistance system 1 (AR1) is activated in slightly acidic media (pH 5.5) in the absence of extracellular glucose or amino acids. *E. coli* grown aerobically under these conditions retain viability under acid challenges as low as pH = 2.5 [54]. This

system is also referred to as the oxidative or glucose-repressed system, since the expression of this system is thought to be regulated either directly or indirectly by RpoS and cyclicAMP receptor protein (CRP) [55,56]. Acid resistance system 2 (AR2) is activated in *E. coli* grown in aerobic conditions in acidic complex media. This system requires the presence of extracellular glucose and glutamate and is dependent upon genes encoding glutamate decarboxylase (*gadAB*) and a glutamate:GABA antiporter (*gabC*) [30]. Under such conditions, *E. coli* have been demonstrated to exhibit acidic resistance up to a pH of 2. The mechanism involves the expenditure of excess cytoplasmic protons during amino acid decarboxylation reactions (see Figure 2), thus raising the internal pH [54,55]. Acid resistance system 3 (AR3) parallels the mechanisms of AR2 with several slight deviations [30,54,55]. AR3 is activated under anaerobic conditions, in complex media with added glucose. It also involves amino acid decarboxylation reactions to lower the internal pH, but requires extracellular arginine in place of glutamate. AR3 also requires increased expression of arginine decarboxylase and an arginine: agmatine antiporter for increased acid tolerance.

Finally, several general acid tolerance mechanisms that regulate the physical properties of the membrane or the effectiveness of ion transport have been identified. These active responses, or those that occur as a result of the cell's ability to sense pH changes, are independent of growth and are induced by pH shifts as small as 0.2 pH units [57]. The first response is the ability of the microorganism to adjust membrane properties, such as lipid content, thus effectively changing the proton permeability [57]. Another cellular response to acid shock is the induction of genes responsible for repairing and preventing lethal cellular damage. Specifically, increased expression of the *oxyR* and *soxR* regulatory genes has been observed by transcriptional profiling of acid tolerant phenotypes [45,58]. These systems regulate the removal of damaging oxidizing agents, thus preventing further DNA damage under acidic stress [46]. Finally, acid tolerance can be achieved by adjusting the ionic transporter efficiency, effectively regulating the anion and cation balance as a means of maintaining a constant internal pH [47].

Organic Acid Production in E. coli

Metabolic and genetic engineering, directed evolution, and classic strain selection have all been employed in the development of *E. coli* strains that produce building block organic acids, including lactic acid, succinic acid, and 3HP [17,25,59,60]. Improved titers have been achieved due to optimization of fermentation conditions and relavant pathways utilized. However, titer limitations exist when fermentation is carried out in unbuffered media, which allows the pH to acidify due to increased acid concentration. Alternatively large amounts of base titrant are required to raise the pH of the media during the organic acid production leaving the final acid molecule in the undissociated form. Following production under these conditions, large volumes of acid must be added to recover the acid in the protonated form. Metabolic and genetic engineering of acid tolerance into production strains, making fermentation at a pH less than the pKa of the acid produced possible, would circumvent the need for the additional consumption of acid and base titrants, and thus lower the overall production cost. Similarly, engineering strain fitness to increase productivity at a decreased pH would improve productivity and reduce base consumption.

Lactic acid production is one of the most successful examples to date of the engineering of large volume chemical production in *E. coli*. *E. coli* was selected as a favorable host strain due to its ability to consume both pentose and hexose sugars and to generate optically pure L-lactic acid, which is the desired product for downstream polylactic acid (PLA) production [61,62]. An effective lactic acid producing strain of *E. coli* was created by induced expression of the L-specific lactic acid dehydrogenase (LDH) gene from *Streptococcus bovis*. High titers (50–75 g/L) were observed under controlled pH (pH = 7) and anaerobic conditions. Titers were drastically decreased (10–20 g/L) as the pH was allowed to drop with increasing acid production [59]. However, allowing the pH to fall below the pKa of lactic acid also resulted in decreased concentration of the acid in the undissociated form, which facilitated the subsequent isolation of the protonated acid. Interestingly, the choice of host strain made a significant difference in lactic acid production [59]. Those constructed from an *E. coli* B strain showed a titer of almost twice that produced from K12 derivatives. The increased production was attributed primarily to differences in the native growth characteristics rather than increased acid tolerance.

Economically competitive titers of succinic acid have also been achieved in *E. coli*. Strains were engineered to limit flux to other anaerobic byproducts normally formed during fermentation [60]. Specifically, succinic acid production was optimized by redirecting the metabolic flux at the pyruvate node away from lactate and formate through inactivation of the pyruvate-formatelyase and lactate dehydrogenase [60,63]. The maximum yield in succinic acid production was approximately 50 g/L in pH controlled cultures. However, similar to lactic acid studies, succinic acid production was significantly repressed when pH was not kept at neutral levels.

A final example of metabolic engineering organic acid production in *E. coli* was reported by Cargill in 2001 [17]. Suthers and Cameron engineered a 2-step glycerol to 3HP

pathway in *E. coli*. Glycerol was first converted to 3HPA via a glycerol dehydratase enzyme (*dhaB* – isolated from *Klebsiella pneumoniae*). 3HPA was then converted to 3HP via an aldehyde dehydrogenase (*ald*). This first pathway was not ideal for several reasons including a very low reported titer (0.2 g/L), the use of the more expensive glycerol as opposed to glucose, and the generation of the highly toxic 3-HPA (reuterin) compound. Selifinova et al. later proposed five additional pathways for the production of 3-HP directly from glucose in *E. coli* [36]. Results for each of such pathways have yet to be reported. One issue that has yet to be addressed is how to fulfill the desire to produce 3-HP at a pH below the pKa = 4.51 of 3-HP, which would lessen the dependency on large volumes of base titrant to retain neutral pH at high titers.

Metabolic engineering of *E. coli* organic acid tolerance represents an important future opportunity. As discussed above, *E. coli* possess several systems for surviving pH as low as 2.0, which is much lower than what is required for an economical biorefining process. Since induction of these systems is well characterized and the relevant genes are known in many cases, future efforts might be better focused on the development of multi-stage fermentations that allow for generation of biomass prior to induction of acid tolerance and, ultimately, acid production. Future genetic engineering efforts might focus on engineering tolerance against the less well characterized metabolic effects associated with increased organic acid anion concentrations. For example, the addition of acetate, benzoate, and propionate to culture media at a concentration of 8 mM has been observed to inhibit growth of *E. coli* up to 50% [35]. The acetate inhibition is thought to be caused by limited methionine pools combined with increasing concentrations of homocysteine, a toxic intermediate, due to inactivation of a key enzyme in the methionine synthesis pathway, which can be countered by the addition of methionine to the media. This finding established that growth inhibition is the result of both of lowered pH and specific anionic effects, which decreases the activity of key enzymes. Thus, engineering tolerance to specific organic acid anion effects by increased expression of inhibited enzymes could aid in increasing overall process productivity.

Conclusion

Organic acids are a valuable sector of the industrial chemical market, which have already been successfully produced through microbial fermentation. However, product titers have been variable, ranging from less than 1 g/L to concentrations cost competitive with current petrochemical production processes. These fermentation processes have been limited in *E. coli* due to product and intermediate toxicity. Toxicity is directly measured by growth inhibition, which specifically decreases productivity. This review highlighted what is known about organic-acid toxicity and tolerance mechanisms in *E. coli*. Specifically, *E. coli* are growth inhibited by the increase in both proton and associated anion concentrations that are characteristic of organic-acid production processes. While several acid-tolerance mechanisms have been characterized in *E. coli*, anion specific mechanisms require additional study. Thus, future metabolic engineering efforts that seek to improve understanding of these issues within the context of organic-acid biorefining applications should prove useful.

Additional material

Additional File 1
Table 1: Organic acids for platform biorefining applications. (see references [64,65])*
Click here for file
[http://www.biomedcentral.com/content/supplementary/1475-2859-4-25-S1.doc]

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United States Patent [19]

Ivey et al.

[11] **Patent Number: 5,928,686**

[45] **Date of Patent: Jul. 27, 1999**

[54] **NUTRIENT FORMULATION AND PROCESS FOR FEEDING YOUNG POULTRY AND OTHER ANIMALS**

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[21] Appl. No.: **08/483,297**

[22] Filed: **Jun. 7, 1995**

[51] **Int. Cl.**^{6} **A23K 1/00; A23K 1/18**

[52] **U.S. Cl.** **426/2**; 426/66; 426/573; 426/575; 426/623; 426/807; 424/442

[58] **Field of Search** 426/2, 807, 66, 426/573, 575, 623; 424/442

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(List continued on next page.)

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[57] **ABSTRACT**

A nutrient formulation including moisture which is designed for use in very young poultry, and a method of feeding it which improves subsequent livability, cumulative feed efficiency and weight gain is disclosed. The method includes feeding a high moisture solid containing at least about 20% by weight water to the poultry before the poultry is allowed to eat dry food ad libitum.

64 Claims, No Drawings

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Wyatt et al. "Influence of Hatcher Holding Times on Several Physiological Parameters Associated With the Immune System of Chickens" Poultry Science, vol. 65 (1986) pp. 2156–2164

Wyatt et al. "Influence of Egg Size, Eggshell Quality, and Posthatch Holding Time on Broiler Performance" Poultry Science, vol. 64 (1985) pp. 2049–2055.

"Cocci Control By Vaccine", From a talk by Dr. Eng Hong Lee at the 1991 Saskatchewan Poultry Industry Conference, Canada Poultryman, Sep. 1991, p. 39.

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NUTRIENT FORMULATION AND PROCESS FOR FEEDING YOUNG POULTRY AND OTHER ANIMALS

BACKGROUND OF THE INVENTION

The present invention is directed to a high moisture solid for providing nutrients, drugs, vitamins, minerals, bile salts, surfactants, probiotics, enzymes, peptides, hormones, prostaglandins, antioxidants, living cells, and immunoactive agents to poultry, and more particularly, a high moisture solid and process which may be used to improve the health and enhance the livability, cumulative weight gain and feed conversion efficiency of poultry and other animals.

For economic reasons, the management of chick hatching in commercial facilities places high importance on percent chicks hatched of eggs set. To achieve hatch rates of 90%, early-hatching birds are often left in the hatch incubator for a period of time to allow the later-hatching chicks to emerge and dry. By the time the chicks are removed from the incubator tray, therefore, they will range in age from several hours up to about 2 days in age (as measured from hatching for each bird). This period is referred to as the post-hatch holding period.

After the chicks are removed from the incubator trays in a commercial hatchery, they are processed (inoculated and sexed) and then placed in boxes commonly referred to as chick boxes for shipping to the production farm. The processing period typically requires several hours and the chicks may reside in the chick boxes for several more hours before transit to the production farm actually begins.

Commercial hatcheries for poultry typically provide chicks for a number of production farms, often over a wide geographical area. Feed and water are not provided until the birds reach the production farm and, as a result, the birds may go several days before feed and water are provided. The presence of the lipid-rich residual yolk sac and reserves of lipid in the liver, however, ensure that the minimal nutritional needs of hatchling birds are met (Freeman et al., *Development of the Avian Embryo*, London, Chapman and Hall, 1974). Thus, a period of inanition after hatching is normal in birds and does not necessarily threaten their survival (Entenman et al., The Lipid Content of Blood, Liver, and Yolk Sac of the Newly Hatched Chick and the Changes That Occur in These Tissues During the First Month of Life, J. Biol Chem., Vol. 133, pp. 231–241 (1940); Vanheel et al., Resorption of Yolk Lipids by the Pigeon Embryo, Comp. Biochem. Physiol., Vol. 68A pp. 641–646 (1981); Phelps et al., The Posthatch Physiology of the Turkey Poult-III. Yolk Depletion and Serum Metabolites, Comp. Biochem. Physiol., Vol. 87A, No. 2 pp. 409–415 (1987); Noble et al., Lipid Changes in the Residual Yolk and Liver of the Chick Immediately after Hatching, Biol Neonate, Vol. 56, pp. 228–236 (1989); Chamblee et al, Yolk Sac Absorption and Initiation of Growth in Broilers, Poultry Science, Vol. 72, pp. 1811–1816 (1992)). This does not mean, however, that using yolk residue as the single nutrient source to hatchlings will provide optimum subsequent livability, disease resistance, or gain and feed efficiency. The delayed placement has been shown to reduce subsequent livability (Kingston, Some Hatchery Factors Involved in Early Chick Mortality, Australian Veterinary Jour., Vol. 55, pp. 418–421 (1979), Fanguy et al., Effect of Delayed Placement on Mortality and Growth Performance of Commercial Broilers, Poultry Science, Vol. 59, pp. 1215–1220 (1980)), disease resistance (Wyatt et al., Influence of Hatcher Holding Times on Several Physiological Parameters Associated

With the Immune System of Chickens, Poultry Science, Vol. 65, pp. 2156–2164 (1986); Casteel et al., The Influence of Extended Posthatch Holding Time and Placement Density on Broiler Performance, Poultry Science, Vol. 73, pp. 1679–1684 (1994)) and growth performance (Hager et al., Education and Production Posthatch Incubation Time and Early Growth of Broiler Chickens, Poultry Science, Vol. 62, pp. 247–254 (1983); Wyatt et al., Influence of Egg Size, Eggshell Quality, and Posthatch Holding Time on Broiler Performance, Poultry Science, Vol. 64, pp. 2049–2055 (1985); Pinchasov et al., Comparison of Post-Hatch Holding Time and Subsequent Early Performance of Broiler Chicks and Turkey Poults, British Poultry Science, Vol. 34, pp. 111–120 (1993)). Provision of individual nutrients such as glucose has not been found to consistently or permanently improve performance or livability when administered as a simple solution in the absence of other nutrients (Azahan et al., Growth, Food Intake and Energy Balance of Layer and Broiler Chickens Offered Glucose in the Drinking Water and the Effect of Dietary Protein Content, British Poultry Science, Vol. 30, pp. 907–917 (1989); Moran, Effects of Posthatch Glucose on Poults Fed and Fasted During Yolk Sac Depletion, Poultry Science, Vol. 68, pp. 1141–1147 (1989); Moran Effects of Egg Weight, Glucose Administration at Hatch, and Delayed Access to Food and Water on the Poult at 2 Weeks of Age, Poultry Science, Vol. 69, pp. 1718–1723 (1990)).

Although provision of water and feed can result in performance superior to that of fasted, water-deprived birds, it is not feasible to include water in the hatch incubator or in transport boxes. This is because humidity control and relatively high temperature are critical in ensuring high hatchability and because presence of water in transport boxes would result in some chicks getting wet. Chicks cannot regulate their body temperature sufficiently well to tolerate evaporation. Since inanition does not threaten survival, commercial practice is not to offer feed or water until the animals reach the farm.

SUMMARY OF THE INVENTION

Among the objects of the invention, therefore, may be noted the provision of a formulation to improve the health and enhance the livability, cumulative weight gain and feed conversion efficiency of poultry and other animals. The formulation may be fed, for example, immediately after hatching of the animal and for this application, the formulation preferably excludes nutrients which are not used well during the first days of life and provides those which are readily used and confer a performance advantage. Also among the objects of the invention is a formulation which contains an amount of water designed to provide adequate moisture during this period. The formulation may contain a source of fatty acids, amino acids, carbohydrate or other ingredients to provide other advantages.

Briefly, therefore, the present invention is directed to a process for enhancing the health, livability, cumulative weight gain or feed conversion efficiency of poultry. The process comprises feeding the hatchlings a high moisture solid before they are started on a diet comprising dry food. The hatchlings are fed the high moisture solid beginning at a point in time preferably within the first 5 days of hatching, more preferably within the first 3 days of hatching, and most preferably as soon as possible after hatching.

The present invention is also directed to a composition and process for inoculating poultry and other animals with living cells such as yeast or bacteria. The animal is fed a high

moisture solid which contains a number of colony forming units of the cells which is sufficient to inoculate the animal and produce the desired effect.

The present invention is further directed to a high moisture solid for improving the health, livability, cumulative weight gain or feed conversion efficiency of poultry. The high moisture solid may comprise, for example, between about 50% and about 95% by weight water, between about 5% and about 50% by weight dry matter, and an additive selected from the group consisting of bile salts, surfactants, enzymes, enzyme co-factors, hormones, prostaglandins, peptides, immunoglobulins, cytokines, antioxidants, amino acids and sources of amino acids and amino acid analogs, antibiotics, vitamins and minerals. The dry matter preferably comprises about 10% to about 90% by weight carbohydrate.

Other objects and features of the invention will be in part apparent and in part pointed out hereinafter.

DETAILED DESCRIPTION OF THE INVENTION

Surprisingly, it has been discovered that the growth of poultry can be stimulated, the livability, cumulative weight gain and feed conversion efficiency of the poultry can be improved by feeding to poultry and other animals a high moisture solid of the present invention. In addition, various substances can be administered by including the substance in the high moisture solid.

In one embodiment of the present invention, the high moisture solid is first fed to hatchlings which are within five, four, three, two or even one day of hatching (as determined for each bird). Preferably, the high moisture solid is fed to the hatchlings before they are fed dry food or allowed to drink water ad libitum, and more preferably before they are fed solid food, at all. The high moisture solid may be placed, for example, in the hatching incubator along with the eggs from which the poultry will hatch so that the high moisture solid is available to the hatchlings immediately upon hatching. Providing the high moisture solid to the chicks prior to their introduction to solid food reduces the likelihood that the hatchlings will suffer by consuming dry food without simultaneously drinking.

In another embodiment of the present invention, the high moisture solid may be made available to the hatchlings prior to or during shipping by placing the high moisture solid in the chick boxes along with the chicks. In accordance with this embodiment, it is preferred that the high moisture solid be placed in the chick boxes before transit begins so that the chicks will have the opportunity to consume the high moisture solid before they begin travelling (that is, moving by surface or air transportation from the site of the incubator to a remote location such as a poultry production farm which may be, for example, one or more miles away from the location of the incubator). The amount of high moisture solid placed in the chick boxes need not be sufficient to enable the chicks to feed on it for the entire transit period.

In a further embodiment of the present invention, the high moisture solid is fed to the poultry after they are shipped from the site where they are hatched to a remote location such as a poultry production farm or other intermediate facility. After being shipped, some chicks do not readily begin eating dry food and drinking water when it is offered. For such applications, it may be desirable to feed the transported poultry the high moisture solid until the poultry begin eating dry food and drinking water ad libitum. In addition, the high moisture solid may also be fed to the poultry at this time or even a later time to administer drugs or other substances as described herein.

The high moisture solid contains at least about 20% by weight (an amount which is in excess of the amount of water contained in "dry" poultry feeds), preferably at least about 25% by weight, still more preferably at least about 30% by weight, still more preferably between about 50% and about 95% by weight, and most preferably between about 65% and about 75% by weight water, based upon the weight of the high moisture solid. The high moisture solid additionally contains at least about 5%, preferably at least about 10%, more preferably about 15% to about 50%, and most preferably between about 25% and about 35% by weight dry matter, based upon the weight of the high moisture solid. The non-aqueous fraction of the high moisture solid is referred to herein as the "dry matter" or the "solid matter" fraction, with the two terms being used interchangeably. The dry matter fraction of the high moisture solid preferably contains carbohydrate and optionally contains other constituents which increase the nutritional value of the high moisture solid or otherwise improve the health of the poultry or other animals.

Carbohydrates provide a source of nutrition for the animals and, in addition, can aid in the formation of the solid. In general, therefore, carbohydrates constitute at least about 5%, preferably between about 10% and about 90%, more preferably between about 50% and about 70%, and most preferably about 60% by weight of the dry matter. The carbohydrates contemplated herein include isolated carbohydrates such as corn starch, potato starch, wheat starch, rice starch, cellulose, pectin, agarose, and gums; bioavailable sugars such as glucose, fructose, and sucrose; chemically modified starches such as modified corn starch, methylcellulose, carboxymethylcellulose, and dextrin; and ground complex carbohydrates such as corn, rice, oats, barley, wheat, sorghum, rye, millet, cassava, triticale and tapioca, in whole, ground, cracked, milled, rolled, extruded, pelleted, defatted, dehydrated, solvent extracted or other processed form. Gums which may be used herein are generally high molecular weight molecules of plant or animal origin, usually with colloidal properties, which in appropriate solvents are able to produce gels, such as agar, algin and carrageenan derived from seaweeds, plant exudates such as gum arabic, ghatti and tragacanth, plant extracts such as pectin, plant seeds such as guar, locust bean, and animal exudates such as plasma, serum albumin, egg albumin, chitin and gelatin. Other gums include amylose and amylopectin and gums of bacterial origin.

The high moisture solid may be formed by mixing the ingredients and heating the mixture. In one embodiment, the mixture contains starch and is heated until the starch granules rupture and the mixture becomes viscous. See, for example, Lewis U.S. Pat. No. 2,593,577. In another embodiment, a gum is dissolved in water at a temperature in excess of about 180° C. to form a colloidal solution which forms a high moisture solid upon cooling. See, for example, U.S. Pat. No. 5,217,740. In yet another embodiment, a gelatinizing aid such as carboxymethylcellulose, lignin, or a lignin derivative is dissolved in water to form a colloidal solution which forms a high moisture solid upon cooling.

To increase the nutritional value of the high moisture solid, the dry matter may contain up to about 70%, preferably between about 15% and about 50% by weight amino acids, precursors or analogues of amino acids, or proteins. Exemplary amino acids are essential amino acids such as methionine, tryptophan, threonine, arginine and lysine. Exemplary amino acid precursors are 2-hydroxy-4-(methylthio)butanoic acid sold, example under the trademark Alimet® by Novus International (St. Louis, Mo.), and

salts of 2-hydroxy-4-(methylthio)butanoic acid such as the calcium and sodium salts. Exemplary proteins include single cell proteins or hydrolysates of proteins such as those from yeast, algae or bacteria; isolated animal proteins, peptides or hydrolysates of proteins such as hemoglobin, myosin, plasma, or other serum proteins, collagen, casein, albumin or keratin; complex protein sources or hydrolysates of proteins such as milk, blood, whey, blood meal, meatmeal, feathermeal, fishmeal, meat and bone meal, poultry offal, poultry by-product meal, hatchery by-products, egg offal, egg white, egg yolk, and eggs without shells; plant protein or hydrolysate of proteins such as soybean meal, isolated soybean protein, wheat protein, wheat germ, distillers grains and gluten.

Fat may also be included in the high moisture solid to increase its nutritional value. The dry matter may, for example, contain up to about 15%, preferably between about 0% and about 10% by weight fat, and more preferably between about 2% and about 5% by weight fat. Suitable fats include fatty acids such as linoleic acid, isolated plant oils such as sunflower, safflower, soybean, peanut, canola, corn, rapeseed, olive, linseed and palmkernal; fat meals such as cottonseed, peanut, rapeseed, palmmeal and nut meals; and fats of animal origin such as lard, butter, poultry fat, tallow and fishoil.

To enable hatchlings to more effectively utilize any fats which may be present in the high moisture solid or to enable the hatchlings to more effectively utilize its yolk-based lipid and protein, the high moisture solid may contain bile salts, cholesterol, surfactants, emulsifying agents, micelles, or an enzyme such as lipase, amylase, maltase, pepsin, trypsin, or other enzyme which commonly occur in the gastrointestinal system, or an enzyme such as keratinase which is not typically found in the gastrointestinal system but which has useful activities. The concentration of the digestion aid will depend upon the application but, in general, will be between about 0.01% and about 5% by weight of the dry matter.

The high moisture solid may additionally contain vitamins and minerals. Vitamin additives may be selected, for example, from vitamin A, B12, biotin, choline, folacin, niacin, pantothenic acid, pyridoxine, riboflavin, thiamin, C, D, 25-hydroxy D, E, and K. Mineral additives may be selected, for example, from calcium, phosphorous, selenium, chlorine, magnesium, potassium, sodium, copper, iodine, iron, manganese and chromium pincolinate. The concentration of the vitamins and minerals will depend upon the application but, in general, will be between about 0.01% and about 5% by weight of the dry matter.

Bacterial, yeast or mold preparations, commonly referred to as probiotics or direct fed microbials, have been administered orally or added to animal feeds to provide various benefits such as altering the gastrointestinal microflora/microbiota of poultry and other animals. Those microbial additives which have been approved for use are identified in the annual Feed Additive Compendium published by The Miller Publishing Company (Minnetonka, Minn.) in cooperation with The Animal Health Institute and the Direct-fed Microbial, Enzyme and Forage Additive Compendium published by The Miller Publishing Company. Among the direct-fed microbials which have been approved are strains of the lactic acid bacteria, particularly those classified in the following genera: Lactobacillus, Lactococcus, and Enterococcus. Included among these are the following species: *Lactobacillus reuteri, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus planturum, Lactobacillus casei, Lactobacillus lactis, Lactococcus lactis, Lactococcus thermophilus, Lactococcus diacetylactis*, and *Enterococcus*

faecium. In addition to these lactic acid bacteria, some species of Bacillus (such as *Bacillus subtilis* and *Bacillus toyoi*), some species of Streptococcus (such as *Streptococcus faecium*), and yeasts and molds (such as *Saccharomyces cerevisiae, Aspergillus oryzae*, and Torulopsis sp.) have also been used as direct fed microbials.

The high moisture solid of the present invention, therefore, may be used as a vehicle to administer direct-fed microbials to poultry and other animals. When used for this purpose, the high moisture solid should contain sufficient colony forming units of the yeast or bacterium to be of benefit to the animal. In general, the high moisture solid used as a direct fed microbial should contain at least about 10^2, preferably about 10^4, and more preferably about 10^6 colony forming units of bacteria or at least about 10, preferably about 10^2, and more preferably about 10^4 colony forming units of yeast per gram of composition. The yeast or bacterium may be incorporated into the high moisture solid prior to solidification or it may be deposited on or in the high moisture solid after it has solidified. Although the high moisture solid may be fed at anytime to alter the gastrointestinal microflora/microbiota of or provide other benefits to the animal, it is preferably fed to poultry as soon as possible after hatching to establish the direct fed microorganism as the dominant microorganism in the gastrointestinal tract and thereby exclude potential pathogens.

The high moisture solid may additionally be used as a vehicle to deliver a variety of other substances to poultry and other animals. For example, the high moisture solid may contain a peptide such as epidermal growth factor, transforming growth factor, granulocyte-macrophage colony stimulating factor, erythropoietin, bombesin, fibroblast growth factor, keratinocyte growth factor, nerve growth factor, vascular endothelial growth factor, bovine or other somatotropin or insulin-like growth factor (IGF-I or IGF-II). The high moisture solid may also contain a steroid or polypeptide hormone such as, estrogen, glucocorticoids, insulin, glucagon, gastrin, calcitonin or somatotropin. The high moisture solid may further contain an antibiotic approved for use in animal feed such as bacitracin, BMD (bacitracin methylenedisalicylate), lincomycin, or virginiamycin or other therapeutic drug. The high moisture solid may also additionally contain a natural or synthetic antioxidant such as ethoxyquin, tocopherol, BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), vitamin C or glutathione; a receptor, transfer factor, chelator or complexing agent which modifies release rates of nutrients or other bioactive compounds; an immunoactive agent such as immunoglobulins, cytokines, antigens, killed cells, attenuated strains, toxins, adjuvants or vaccines; or a palatability modifier such as food coloring, grit, oyster shell, whole seeds or grains. The concentration of these additives will depend upon the application but, in general, will be between about 0.1% and about 10% by weight of the dry matter.

In a preferred embodiment, the high moisture solid contains about 65–75% by weight water with the nutrients being protein (35%), carbohydrate (60%) and fat (5%), based upon the weight of the solid matter fraction of the high moisture solid. High moisture solids having this nutrient profile may be prepared, for example, from the following ingredients mix (based upon the weight of the solids):

soybean meal	30%
egg white	8%
corn starch	4%
corn meal	30%
Alimet ®	0.5%
propionic acid	0.5%
citric acid	to pH 4.5–6

High moisture solids containing these ingredients (and optionally one or more of the above-identified additives) can be made by dry mixing the ingredients, adding hot water (80° C.) and quickly mixing the wetted ingredients while maintaining the temperature above the starch gelation temperature for at least one minute. The mixture is then stirred and pressed into a dish, cylinder or other mold.

Alternatively, the high moisture solid may be prepared from a poultry starter diet formulation. These formulations specify minima for protein, energy, vitamins and other nutrients. The simplest high moisture solid formulation of the present invention thus consists of about 30 parts by weight starter feed and about 70 parts by weight water, heated to the gelation temperature of corn starch. Starter feeds typically contain about 3200 Kcal/kg with the nutrient profile being about 20–25 wt. % protein, about 5–8 wt. % fat, about 3–5 wt. % vitamins and minerals, and a balance of carbohydrate. Furthermore, it is believed that such a formulation can be improved upon through selective replacement of ingredients less available to the hatchling with ingredients of higher digestibility at this age, such as hydrolyzed proteins. Alternatively, the digestibility of ingredients could be improved with additions to the formulation such as, but not limited to, enzymes, bile salts or surfactants. Similarly, overall performance may be improved with the addition of selected micro ingredients, minerals, microorganisms, growth promotants, hormones, prostaglandins such as E_2 or other factors which promote enhanced digestive enzyme activity, nutrient absorption or maturation of the gastrointestinal system as a whole.

In general, highly available protein sources might include hydrolyzed poultry protein, hydrolyzed casein, or peptone. In contrast, less available protein sources such as by-product meals or vegetable proteins might be fed in combination with factors such as proteases or microorganisms that secrete proteases to increase digestibility. Similarly, carbohydrates such as glucose may be chosen for high availability, or more complex sources such as ground corn or potato starch may be supplemented with enzymes or subjected to gelation to increase their availability. Digestibility of saturated fats could be improved through the addition of lipase, bile salts or surfactants. Thus, the formulation would include either highly available ingredients or additives or handling methods which improve their digestion in very young birds. The ingredients would be administered in a high moisture semi-solid or solid form.

In addition, it has been demonstrated that the gastrointestinal system of young birds is not able to use certain ingredients such as tallow with the same efficiency as mature birds (Freddie et al., Factors Affecting the Absorbability of Certain Dietary Fats in the Chick, J. Nutrition, Vol. 70, pp. 447–452 (1960); Gomez et al., The Use of Bile Salts to Improve Absorption of Tallow in Chicks, One to Three Weeks of Age, Poultry Science Vol. 55, pp. 2189–2195 (1976); Polin et al., The Effect of Bile Acids and Lipase on Absorption of Tallow in Young Chicks, Poultry Science, Vol. 59, pp. 2738–2743 (1980); Sell et al., Influence of Age on Utilization of Supplemental Fats by Young Turkeys, Poultry Science, Vol. 65, pp. 546–554 (1986)). Ontogenetic changes which accompany improved digestion include increased levels of pancreatic and intestinal enzymes (Krogdahl et al., Influence of Age on Lipase, Amylase, and Protease Activities in Pancreatic Tissue and Intestinal Contents of Young Turkeys, Poultry Science, Vol. 68, pp. 1561–1568 (1989); Sell et al., Intestinal Disaccharidases of Young Turkeys: Temporal Development and Influence of Diet Composition, Poultry Science, Vol. 68, pp. 265–277 (1989); Noy et al., Digestion and Absorption in the Young Chick, Poultry Science, Vol. 74, pp. 366–373 (1995)), overall gut surface area for absorption (Nitsan et al., Growth and Development of the Digestive Organs and Some Enzymes in Broiler Chicks After Hatching, British Poultry Science, Vol. 32, pp. 515–523 (1991); Nitsan et al., Organ Growth and Digestive Enzyme Levels to Fifteen Days of Age in Lines of Chickens Differing in Body Weight, Poultry Science, Vol. 70, pp. 2040–2048 (1991); Sell et al., Developmental Patterns of Selected Characteristics of the Gastrointestinal Tract of Young Turkeys, Poultry Science, Vol. 70, pp. 1200–1205 (1991)), and changes in nutrient transporters (Shehata et al., Development of Brush-Border Membrane Hexose Transport System in Chick Jejunum, Am. J. Physiol, Vol. 240, pp. G102–G108 (1981); Buddington et al., Ontogenetic Development of Intestinal Nutrient Transporters, Annu. Rev. Physiol., Vol. 51, pp. 601–619 (1989); Moreto et al., Transport of L-Proline and α-Methyl-D-Glucoside by Chicken Proximal Cecum During Development, Am. J. Physiol, Vol. 260, pp. G457–G463 (1991)). The high moisture solid of the present invention would minimize or exclude poorly used ingredients and substitute more highly available ingredients as assessed by subsequent bird performance.

The composition of the high moisture solid may also be tailored to meet environmental conditions. Hatchers and brooders are typically warm, relatively low humidity environments. Under these conditions, high moisture solids containing simple starch, protein and water can rather quickly become leather-like or very hard upon dehydration, thus making it difficult for the hatchlings to consume the solid. Materials such as finely ground corn, modified corn starches, and carboxymethylcellulose tend to improve water retention and long-term texture (24–48 hr.), but may result in the formulation sticking to the bird. For this environment, therefore, the high moisture solid preferably comprises about 30–40% by weight dry matter with the dry matter comprising coarser grain and protein meals and a coagulated protein such as egg white.

In contrast, when birds are packed into chick transit boxes used by commercial hatcheries at the usual commercial density (24 birds per quarter) air flow is very low. In addition, hatchlings lose significant amounts of water, particularly during the first 24 hours after hatching. This combination of higher humidity, lower air flow and greater population density can result in mortality if the birds are wetted by the high moisture solid. For this environment, therefore, the high moisture solid comprises about 30 to 40% by weight dry matter with the dry matter comprising a gum or modified corn starch.

The quantity of the high moisture solid fed will be a function of the animal species, age, environmental conditions such as temperature and humidity and, in the case of poultry, the length of the preplacement period, i.e., the total time consumed in the post-hatch holding period, the processing period and in transit to the poultry production farm. In general, however, at least about 10 grams of high moisture solid per chick per day should be provided to 0 to 2 day old chicks, about 20 grams of high moisture solid per chick per day should be provided to 2 to 3 day old chicks, and up to

about 50 grams of high moisture solid per chick per day should be provided to 4 to 7 day old chicks.

As previously noted, chicks conventionally are placed with poultry production farms within about 2 days of hatching. This practice has developed, in part, out of the fact that hatchers typically do not provide food or water to the hatchlings and the fact that the hatchlings must receive water and a source of nutrition by about 3 days or else they suffer. Because the composition of the high moisture solids of the present invention can be controlled to meet the changing nutritional requirements of the hatchlings as they mature, it may become practical for hatchers to delay sending chicks to poultry production farms for a longer period of time without experiencing many of the difficulties associated with providing water and nutrition to the chicks. Thus, for example, hatchers could conveniently feed the high moisture composition of the present invention to the chicks for a period of about 3 to about 7 days from hatching before shipping them on to the poultry production farms. Alternatively, the chicks could be shipped from the hatcher to an intermediate facility where they could be fed the high moisture solid for a period of about 7 days and then shipped to the standard poultry production farm. Either approach would allow the poultry production farms to more efficiently utilize their houses without burdening the hatchers with feeding the hatchlings water and dry food.

The following examples will illustrate the invention.

EXAMPLE 1

The performance of 1 to 4 day old birds, i.e., birds which were no less than 1 day old and no more than 4 days old at the start of the test as measured from hatching for each bird, fed high moisture solids consisting of agar (1.5% agar and 98.5% by weight water) or agar and egg yolk (1.5% agar, 10% egg yolk and 88.5% by weight water) were compared to fasted and water deprived birds. The results are presented in Table 1. Birds initially lost weight on all feeding regimens and agar alone gave no benefit in either cumulative gain or cumulative feed-to-gain ratio ("FTG"). Agar plus yolk showed an effect on cumulative gain on days 6 and 13, but cumulative feed-to-gain ratio (sometimes referred to herein as cumulative feed efficiency) was poorer than for fasted birds. The data also suggest that hydration alone (agar treatment) with or without yolk conferred no cumulative feed efficiency benefit in this study. Cumulative livability was improved by feeding either water-containing formulation.

TABLE 1

Growth of Birds Fed Nothing or Formulations Consisting of Agar (1.5%) with and without Egg Yolk (10%)

Trt	Cumul. Gain Day 3	Cumul. Gain Day 6	Cumul. FTG Day 6	Cumul. Gain Day 13	Cumul. FTG Day 13	Cumulative Feed Intake Day 13	Cumulative Livability Day 13
Fasted 24 h	-8.0	25.8 g	1.86	135.6 g	1.60	274 g	94%
Agar	-7.2	32.8 g	1.95	135.7 g	1.63	275 g	98%
Agar & Yolk	-7.8	37.5 g	1.70	139.4 g	1.63	281 g	98%

EXAMPLE 2

In this example, groups of one to four day old birds were fed for 24 or 48 hours a high moisture solid consisting of starter feed and water. Pens were given enough high moisture solid for each bird to consume 10 g. The feed was present at either 25, 50 or 100% of the high moisture solid. From Table 2 it appears that the high moisture solid containing 25% dry matter gave the best cumulative gain after feeding either 24 or 48 hr. It should be noted, however, that all high moisture solids showed cumulative gain superior to the fasted controls. When cumulative feed efficiency was corrected for differences in body weight (BW FTG), the 25% dry matter high moisture solid again was superior to the others whether fed for 24 or 48 hr. Cumulative feed intake subsequent to the 48 hr treatment period was higher when birds were given high moisture solids than when they were fasted. This was the case for formulations containing 25, 50 or 100% dry matter. Cumulative livability data suggest that the high moisture solids containing a greater percentage of dry matter are associated with lower livability than the fasted control or 25% dry matter formulations.

TABLE 2

Growth of Birds Fed Starter Feed and Moisture Combinations

Treatment	Cumul. Gain Day 13	Cumul. FTG Day 13	BW Cumul. FTG Day 13	Cumul. Intake Day 13	Cumulative Livability Day 13
Fasted 24 h	300.9 g	1.336	1.292	398.8 g	100%
Formulation 24 h Dry Matter 25%	303.5 g	1.397	1.285	400.5 g	100%
Formulation 24 h Dry Matter 50%	289.9 g	1.382	1.323	360.9 g	100%
Formulation 24 h Dry Matter 100%	266.7 g	1.392	1.285	375.9 g	94%
Fasted 48 h	222.8 g	1.371	1.372	304.5 g	96%
Formulation 48 h Dry Matter 25%	284.6 g	1.278	1.248	362.5 g	100%
Formulation 48 h Dry Matter 50%	267.9 g	1.383	1.335	360.4 g	83%
Formulation 48 h Dry Matter 100%	225.9 g	1.394	1.380	328.4 g	83%

EXAMPLE 3

In this example, groups of one to four day old birds were given 20 g each of a high moisture solid consisting of gelatin and Alimet® (2-hydroxy-4-(methylthio)butanoic acid) base with additions of either corn starch or corn starch and lysine. The dry matter content of the high moisture solid was about 5% and the amount of each of the dry matter constituents, based upon the weight of the high moisture solid for each formulation, was as indicated in Table 3. The formulation containing corn starch, gelatin and Alimet® showed cumulative gain and livability superior to the fasted control and the other two formulations. Treatments 2 and 3 also showed superior cumulative feed intake when compared with the fasted control, but the formulations tended to liquify at the brooding temperature would could cause problems in brooding and transit boxes.

TABLE 3

Growth of Birds Fed Formulations Containing Starch, Gelatin, Alimet and Lysine Combinations

Trt	Corn Starch	Gelatin	Alimet®	Lysine	Cumul Gain Day 14	Cumul FTG Day 14	Cumul Intake Day 14	Cumulative Livability Day 14
Fasted 24 hr					291.8 g	1.22	356 g	95%
1	0 g	2.5%	.25%	0	290.8 g	1.22	348 g	80%
2	2.5%	2.5%	.25%	0	312.7 g	1.23	392 g	100%
3	2.5%	2.5%	.25%	.15%	286.1 g	1.34	381 g	80%

EXAMPLE 4

Groups of one to four day old birds were fed formulations containing sources of fats and protein administered with and without added lipase to assist in the digestion of the fat. All formulations contained corn starch, Alimet, lysine and the bile salt, chenodeoxycholic acid. In one case, protein and fat were provided together in the form of yolk solids. In the second case, poultry protein and soy oil were used to provide the protein and fat. The dry matter content of the high moisture solid was about 25% and the amount of each of the dry matter constituents, based upon the weight of the high moisture solid for each formulation, was as indicated in Table 4. Table 4 indicates that the improved cumulative gains and cumulative feed efficiencies were observed in all formulation treatments. Lipase did not appear to be enhance the availability of these complex fat sources. Superior early cumulative feed intake was achieved with yolk solids in the absence of additional lipase. It should be noted that yolk was also used in Example 1, but bird response was not evident in the absence of a source of carbohydrates, bile salts, a methionine source and added lysine.

TABLE 4

Growth of Birds Fed Formulations Containing Sources of Protein and Fat, with and without Lipase (Corn starch 2.5%, Alimet .25%, Lysine .05%, Chenodeoxycholic acid .02%)

Trt	Addition	Fat	Protein	Cumul. Gain Day 12	FTG Day 12	Cumul. intake Day 12	Cumulative Livability Day 12
Fasted				282.6 g	1.30	333.2 g	100%
1	Egg Yolk (25%)	7.7%	5.5%	284.4 g	1.22	345.6 g	100%
2	Lipase (2%) Egg Yolk (25%)	7.7%	5.5%	298.5 g	1.24	362.7 g	100%
3	Soy Oil (10%) Poultry Protein (20%)	10%	7.5%	264.5 g	1.25	334.2 g	95%
4	Lipase (2%) Soy Oil (10%) Poultry Protein (20%)	10%	7.5%	253.8%	1.26	312.4 g	100%

EXAMPLE 5

Groups of one to four day old birds fed agar (1.5% agar and 98.5% water) and agar plus a direct fed microbial (1.5% agar, 88.5% water, 10% Biomate direct fed microbial including the microbial carrier) were compared to a fasted control. The direct fed microbial ("DFM") consisted of two species of Lactobacilli and two species of Bacilli. The direct fed microbial contained 2.2×10^8 colony forming units per gram of material for each of the Lactobacilli species and 5.5×10^8 colony forming units per gram of material for each of the Bacilli species with each pen of 8 birds receiving 1 gram of product. Although the cumulative feed efficiency of this treatment was poorer than that of agar alone, cumulative gain appeared to increase in the presence of water and the DFM. The DFM, therefore, provided some benefit on its own and to optimize this effect more nutrients should be added to the high moisture solid.

TABLE 5

Growth of Birds Fed Agar (1.5%) and Agar Containing a Direct Fed Microbial Consisting of *Lactobacillus acidophilus* and *lactis*, and *Bacillus subtilis* and *licheniformis* (10%)

Treatment	Cumulative Gain Day 21	Cumulative Feed to Gain Day 21	Cumulative Feed Intake Day 21	Cumulative Livability Day 21
Fasted 24 h	738.3 g	1.388	985.8 g	97%
Agar (1.5%)	720.8 g	1.386	998.0 g	92%
Agar (1.5%) DFM (10%)	724.3 g	1.387	1003.4 g	92%

EXAMPLE 6

This example shows the response of one to four day old hatchlings to casein, enzyme hydrolyzed casein and casein administered with a source of proteolytic activity. The high moisture solid contained 85% water with a balance of constituents as indicated in Table 6. In treatment 3, 0.6% pepsin (based upon the weight of the high moisture solid) was added to the formulation and in treatment 4, a microbe which secretes a proteolytic enzyme was added. All formulation treatments showed superior cumulative gain, cumulative feed efficiency and livability when compared to the fasted control.

TABLE 6

Growth of Birds Fed Formulations with Casein, Hydrolyzed Casein, Casein with Pepsin or Casein with B. licheniformis (2 x 10^9/ml) (Ground corn: 10%, Agar: .25%, Alimet: .125%, Lysine: .1%)

Trt	Casein	Cumulative Gain Day 12	Cumulative Feed to Gain Day 12	Cumulative Feed Intake Day 12	Cumulative Livability Day 12
Fasted 24 h		207.2 g	1.34	301.4 g	79%
1	Casein (10%)	249.3 g	1.21	301.2 g	92%
2	Hydrolyzed Casein (10%)	234.8 g	1.30	298.3 g	96%
3	Casein (10%) Pepsin (.5%)	234.8 g	1.26	300.2 g	91%
4	Casein (10%) B. licheniformis	248.8 g	1.24	296.0 g	91%

EXAMPLE 7

In this example, zero to two day old birds were fed formulations consisting of 10% dry matter in the form of corn starch (2.5%), protein (5%), and glucose (2.5%), based upon the weight of the high moisture solid. Birds were treated for 24, 48 or 72 hours, to test the possibility of treating birds over the total preplacement period of approximately 2 days in the hatching incubator and 1 day in transit. All formulation treated birds showed cumulative gain superior to birds fasted for an equivalent period. In addition, the birds treated with formulation for 24 and 48 hours also showed superior cumulative feed efficiencies. The response appeared to decline at the 72 hour time point. It appears from these data that 10% dry matter is sufficient to improve performance of young birds over a 2 day period, but that a higher concentration of nutrients may be required by the third day. It should be noted that for each time period, livability of formulation fed birds was superior to fasted controls.

TABLE 7

Growth of Birds Fed Hatchery Formulations Consisting of Corn Starch (2.5%), Porcine Plasma (5%), Agar (.5%), Alimet (.125%), Lysine (.125%), Glucose 2.5% Total 10% Dry Matter.

Treatment	Cumulative Gain Day 16	Cumulative Feed to Gain Day 16	Cumulative Feed Intake Day 16	Cumulative Livability Day 16
Fasted 24 h	405.4 g	1.433	581.1 g	93%
Formulation 24 h	435.5 g	1.422	619.4 g	96%
Fasted 48 h	380.3 g	1.455	553.3 g	95%
Formulation 48 h	391.7 g	1.413	553.5 g	100%
Fasted 72 h	333.3 g	1.420	473.3 g	92%
Formulation 72 h	348.5 g	1.456	507.4 g	93%

In view of the above, it will be seen that the several objects of the invention are achieved.

As various changes could be made in the above compositions and processes without departing from the scope of the invention, it is intended that all matter contained in the above description be interpreted as illustrative and not in a limiting sense.

What is claimed is:

1. A process for enhancing the health, livability, cumulative weight gain, or feed conversion efficiency of poultry, the process comprising making available for consumption ad libitum a high moisture solid to the poultry before the poultry is allowed to eat dry food ad libitum, the high moisture solid containing between about 30% and about 90% by weight water and between about 10% and about 70% dry matter based upon the weight of the high moisture solid,

wherein the dry matter contains at least about 10% by weight carbohydrate and between about 15% and about 50% by weight of an amino acid source based on the weight of the dry matter.

2. The process of claim 1 wherein the high moisture solid contains between about 50% and about 85% by weight water and between about 15% and about 50% by weight dry matter, and the poultry comprises hatchlings which are within 3 days after hatching.

3. The process of claim 1 wherein the poultry is placed in a container for shipment to a poultry farm and the high moisture solid is made available to the poultry for consumption ad libitum prior to placing the poultry in the container.

4. The process of claim 1 wherein the high moisture solid is made available to the poultry by placing the high moisture solid in an incubator along with the eggs from which the poultry will hatch, thereby making the high moisture solid available to the poultry upon hatching.

5. The process of claim 1 wherein the high moisture solid contains at least about 50% by weight water.

6. The process of claim 1 wherein the high moisture solid lacks the complete nutritional requirements of poultry which is between 5 and 10 days of hatching.

7. The process of claim 1 wherein the high moisture solid contains between about 50% and about 75% by weight water and between about 25% and about 50% by weight dry matter with the carbohydrate comprising at least about 50% by weight of the dry matter.

8. The process of claim 7 wherein:

the carbohydrate is selected from the group consisting of corn starch, wheat starch, modified corn starch, a gum, whole, ground, cracked, milled, rolled, extruded, pelleted, defatted, dehydrated, solvent extracted, or other processed form of corn or wheat, and mixtures thereof; and

the amino acid source is selected from the group consisting of methionine, tryptophan, threonine, arginine, lysine, 2-hydroxy-4-(methylthio)butanoic acid, a salt of 2-hydroxy-4-(methylthio)butanoic acid, serum proteins, casein, soybean meal, fishmeal, meat meal, egg white, egg yolk, eggs without shells, and mixtures thereof.

9. The process of claim 8, wherein the gum is algin.

10. The process of claim 1 wherein the high moisture solid additionally contains fat, a vitamin, a mineral, an enzyme, an

enzyme co-factor, a peptide, a hormone, a prostaglandin, an antibiotic, a natural or synthetic antioxidant, yeast, bacteria, a palatability modifier, a digestion aid, an immunoactive agent, or a growth promoter.

11. The process of claim 1 wherein the high moisture solid comprises at least about 10^2 colony forming units of bacteria or at least about 10 colony forming units of yeast per gram of high moisture solid.

12. The process of claim 1 wherein the high moisture solid comprises at least about 10^2 colony forming units of a lactic acid bacterium per gram of high moisture solid.

13. The process of claim 1 wherein the high moisture solid comprises at least about 10^2 colony forming units of a microorganism of the genus Lactobacillus per gram of high moisture solid.

14. The process of claim 1 wherein the high moisture solid comprises at least about 10^2 colony forming units of a microorganism of the genus Bacillus per gram of high moisture solid.

15. The process of claim 1 wherein the high moisture solid is made available to the poultry by placing the high moisture solid along with the poultry in a shipping container.

16. A process for enhancing the health, livability, cumulative weight gain, or feed conversion efficiency of poultry, the process comprising making available for consumption ad libitum a high moisture solid to the poultry before the poultry eats dry food, the high moisture solid containing between about 30% and 90% by weight water and between about 10% and about 70% dry matter based upon the weight of the high moisture solid,

wherein the dry matter contains a nutritive carbohydrate and an amino acid source selected from the group consisting of proteins, complex protein sources, amino acids, precursors of amino acids, and analogs of amino acids, and

wherein the nutritive carbohydrate constitutes at least about 10% by weight of the dry matter.

17. The process of claim 16 further comprising hatching the poultry in one location and shipping the hatchlings to a remote location to be grown, wherein the high moisture solid is made available to the poultry after the poultry has been shipped to the remote location.

18. The process of claim 16 wherein the high moisture solid is made available to the poultry by placing the high moisture solid in an incubator along with the eggs from which the poultry will hatch thereby making the high moisture solid available to the poultry upon hatching.

19. The process of claim 16 wherein the high moisture solid contains at least about 50% to 75% by weight water and about 25% to about 50% by weight dry matter, the dry matter comprising between about 15% and about 50% by weight proteins, complex protein sources amino acids, or precursors or analogues of amino acids, about 50% to about 70% by weight carbohydrate, and between about 0% and about 5% by weight fat.

20. The process of claim 19 wherein:

the carbohydrate is selected from the group consisting of corn starch, wheat starch, modified corn starch, a gum, whole, ground, cracked, milled, rolled, extruded, pelleted, defatted, dehydrated, solvent extracted, or other processed form of corn or wheat, and mixtures thereof; and

the proteins, complex protein sources, amino acids, or precursors or analogues of amino acids are selected from the group consisting of methionine, tryptophan, threonine, arginine, lysine, 2-hydroxy-4-(methylthio) butanoic acid, a salt of 2-hydroxy-4-(methylthio) butanoic acid, serum proteins, casein, soybean meal, fishmeal, meat meal, egg white, egg yolk, eggs without shells, and mixtures thereof.

21. The process of claim 20, wherein the gum is algin.

22. The process of claim 16, wherein the high moisture solid is made available to the poultry by placing the high moisture solid along with the poultry in a shipping container.

23. A process for enhancing the health, livability, cumulative weight gain, or feed conversion efficiency of poultry hatchlings, the process comprising making available to the hatchlings within 5 days after hatching a high moisture solid for consumption ad libitum before the poultry is allowed to eat dry food, the high moisture solid containing between about 50% and 85% by weight water and between about 15% and about 50% by weight dry matter based upon the weight of the high moisture solid,

wherein the dry matter contains a carbohydrate and an amino acid source selected from the group consisting of proteins, complex protein sources, amino acids, precursors of amino acids, and analogs of amino acids,

wherein the carbohydrate constitutes at least about 10% by weight of the dry matter.

24. The process of claim 23 wherein the high moisture solid is made available to the hatchlings within the first 3 days after hatching.

25. The process of claim 23 wherein the high moisture solid is made available to the hatchlings within the first 2 days after hatching.

26. The process of claim 23 wherein the high moisture solid contains between about 50% and about 75% by weight water and between about 25% and about 50% by weight dry matter with the carbohydrate comprising at least about 50% by weight of the dry matter.

27. The process of claim 26 wherein:

the carbohydrate is selected from the group consisting of corn starch, wheat starch, modified corn starch, a gum, whole, ground, cracked, milled, rolled, extruded, pelleted, defatted, dehydrated, solvent extracted, or other processed form of corn or wheat, and mixtures thereof; and

the amino acid source is selected from the group consisting of methionine, tryptophan, threonine, arginine, lysine, 2-hydroxy-4-(methylthio)butanoic acid, a salt of 2-hydroxy-4-(methylthio)butanoic acid, serum proteins, casein, soybean meal, fishmeal, meat meal, egg white, egg yolk, eggs without shells, and mixtures thereof.

28. The process of claim 27 wherein the gum is algin.

29. The process of claim 23 wherein the high moisture solid additionally contains fat, a vitamin, a mineral, an enzyme, an enzyme co-factor, a peptide, a hormone, a prostaglandin, an antibiotic, an antioxidant, yeast, bacteria, a palatability modifier, a digestion aid, an immunoactive agent, or a growth promoter.

30. The process of claim 23 wherein the high moisture solid comprises at least about 10^2 colony forming units of bacteria or at least about 10 colony forming units of yeast per gram of high moisture solid.

31. The process of claim 23, wherein the high moisture solid comprises at least about 10^2 colony forming units of a lactic acid bacterium per gram of high moisture solid.

32. The process of claim 23, wherein the high moisture solid lacks the complete nutritional requirements of such newly hatched poultry which is between 5 and 10 days after hatch.

33. The process of claim 23, wherein the high moisture solid is made available to the poultry by placing the high moisture solid along with the poultry in a shipping container.

34. A process for inoculating poultry with yeast or bacteria, the process comprising making available for consumption ad libitum a high moisture solid to the poultry before the poultry is fed dry food, the high moisture solid containing:

between about 30% and about 90% by weight water, and between about 10% and about 70% by weight dry matter based upon the weight of the high moisture solid,

wherein the dry matter contains at least about 10% by weight carbohydrate and between about 15% and about 50% by weight of an amino acid source, and

at least 10 colony forming units of the yeast or 10^3 colony forming units of the bacteria per gram of the high moisture solid.

35. The process of claim 34 wherein the high moisture solid contains at least about 10^3 colony forming units of a lactic acid bacterium per gram of high moisture solid.

36. The process of claim 34 wherein the high moisture solid comprises at least about 10^3 colony forming units of a microorganism of the genus Lactobacillus per gram of high moisture solid.

37. The process of claim 34 wherein the high moisture solid comprises at least about 10^3 colony forming units of a microorganism of the genus Bacillus per gram of high moisture solid.

38. The process of claim 34 wherein:

the carbohydrate is selected from the group consisting of corn starch, wheat starch, modified corn starch, a gum, whole, ground, cracked, milled, rolled, extruded, pelleted, defatted, dehydrated, solvent extracted, or other processed form of corn or wheat, and mixtures thereof; and

the amino acid source is selected from the group consisting of methionine, tryptophan, threonine, arginine, lysine, 2-hydroxy-4-(methylthio)butanoic acid, a salt of 2-hydroxy-4-(methlthio)butanoic acid, serum proteins, casein, soybean meal, fishmeal, meat meal, egg white, egg yolk, eggs without shells, and mixtures thereof.

39. The process of claim 38 wherein the gum is algin.

40. The process of claim 34 wherein the high moisture solid is made available to the poultry by placing the high moisture solid along with the poultry in a shipping container.

41. A process for hatching poultry eggs, comprising:

(a) placing a set of poultry eggs in an incubator until the poultry hatches from the eggs,

(b) placing the hatchlings in a container for shipment to a remote location,

(c) shipping the hatchlings in the container to a remote location, and

(d) making available for consumption ad libitum a high moisture solid to the hatchlings before they are shipped to the remote location and before they are allowed to eat dry food,

the high moisture solid containing between about 30% and 90% by weight water and between about 10% and about 70% dry matter based upon the weight of the high moisture solid,

wherein the dry matter contains at least about 10% by weight carbohydrate and between about 15% and about 50% by weight of an amino acid source based on the weight of the dry matter.

42. The process of claim 41 wherein the high moisture solid is made available to the hatchlings prior to placing the hatchlings in the shipping container.

43. The process of claim 41 wherein the hatchlings of step (d) are no more than 3 days old.

44. The process of claim 41 wherein:

the carbohydrate is selected from the group consisting of corn starch, wheat starch, modified corn starch, a gum, whole, ground, cracked, milled, rolled, extruded, pelleted, defatted, dehydrated, solvent extracted, or other processed form of corn or wheat, and mixtures thereof; and

the amino acid source is selected from the group consisting of methionine, tryptophan, threonine, arginine, lysine, 2-hydroxy-4-(methylthio)butanoic acid, a salt of 2-hydroxy-4-(methylthio)butanoic acid, serum proteins, casein, soybean meal, fishmeal, meat meal, egg white, egg yolk, eggs without shells, and mixtures thereof.

45. The process of claim 44 wherein the gum is algin.

46. The process of claim 41 wherein the high moisture solid is made available to the poultry by placing the high moisture solid along with the poultry in a shipping container.

47. A process for enhancing the health, livability, cumulative weight gain, or feed conversion efficiency of poultry, the process comprising making available for consumption ad libitum to the poultry before the poultry is allowed to eat dry food ad libitum a high moisture solid containing:

between about 50% and about 95% by weight water and

between about 5% and about 50% by weight dry matter, based on the weight of the high moisture solid,

wherein the dry matter contains between about 10% and about 90% by weight carbohydrate, up to about 70% by weight amino acids, precursors or analogues of amino acids, complex protein sources, or proteins, and up to about 15% by weight fat, based on the weight of the dry matter.

48. The process of claim 47, wherein the high moisture solid contains:

between about 50% and about 95% by weight water and between about 15% and about 50% by weight dry matter, based on the weight of the high moisture solid,

wherein the dry matter contains between about 50% and about 70% by weight carbohydrate, about 15% to about 50% by weight amino acids, precursors or analogues of amino acids, complex protein sources or proteins, and about 0% to about 10% by weight fat, based on the weight of the dry matter.

49. The process of claim 47, wherein the high moisture solid contains:

between about 65% and about 75% by weight water and between about 25% and about 35% by weight dry matter, based on the weight of the high moisture solid,

wherein the dry matter contains about 60% by weight carbohydrate, between about 15% to about 50% by weight amino acids, precursors or analogues of amino acids, complex protein sources or proteins, and about 0% to about 5% by weight fat, based on the weight of the dry matter.

50. The process of claim 49 wherein:

the carbohydrate is selected from the group consisting of corn starch, wheat starch, modified corn starch, a gum, whole, ground, cracked, milled, rolled, extruded, pelleted, defatted, dehydrated, solvent extracted, or other processed form of corn or wheat, and mixtures thereof; and

the amino acids, precursors or analogues of amino acids, complex protein sources, or proteins are selected from the group consisting of methionine, tryptophan, threonine, arginine, lysine, 2-hydroxy-4-(methylthio)

butanoic acid, a salt of 2-hydroxy-4-(methylthio) butanoic acid, serum proteins, casein, soybean meal, fishmeal, meat meal, egg white, egg yolk, eggs without shells, and mixtures thereof.

51. The process of claim 50 wherein the gum is algin.

52. The process of claim 47, wherein the high moisture solid contains,

between about 65% and about 75% by weight water and between about 25% and about 35% by weight dry matter, based on the weight of the high moisture solid,

wherein the dry matter contains about 60% by weight carbohydrate, about 35% by weight protein or complex protein source, and about 5% by weight fat, based on the weight of the dry matter.

53. The process of claim 47, wherein the high moisture solid additionally contains a component selected from the group consisting of a vitamin, a mineral, a receptor, a transfer factor, a chelator, a complexing agent, a palatability modifier, a digestion aid, a steroid, an immunoactive agent, a direct fed microbial, an enzyme co-factor, a peptide, a hormone, a prostaglandin, an antibiotic, a natural or synthetic antioxidant, and a growth promoter.

54. The process of claim 47 wherein the poultry are within 5 days after hatching.

55. The process of claim 47 wherein the poultry are within 3 days after hatching.

56. The process of claim 47 wherein the poultry are within 2 days after hatching.

57. The process of claim 47 wherein the high moisture solid is made available to the poultry by placing the high moisture solid along with the poultry in a shipping container.

58. A process for hatching poultry eggs comprising:

(a) placing a set of poultry eggs in an incubator until the poultry hatches from the eggs,

(b) placing the hatchlings in a container for shipment to a remote location,

(c) shipping the hatchlings in the container to a remote location, and

(d) making available for consumption ad libitum to the hatchlings before they are shipped to the remote location and before they are fed dry feed a high moisture solid containing,

between about 50% and about 95% by weight water and

between about 5% and about 50% by weight dry matter, based on the weight of the high moisture solid,

wherein the dry matter contains between about 10% and about 90% by weight carbohydrate, up to about 70% by weight amino acids, precursors or analogues of amino acids, complex protein sources, or proteins, and up to about 15% by weight fat, based on the weight of the dry matter.

59. The process of claim 58 wherein the high moisture solid additionally contains a component selected from the group consisting of a vitamin, a mineral, an enzyme co-factor, a peptide, a hormone, a prostaglandin, an antibiotic, a natural or synthetic antioxidant, a bacterium, a yeast, a palatability modifier, a digestion aid, an immunoactive agent, and a growth promoter.

60. The process of claim 58 wherein the hatchlings are fed the high moisture solid prior to being placed in the shipping container.

61. The process of claim 58 wherein the hatchlings of step (d) are no more than 5 days old.

62. The process of claim 58 wherein:

the carbohydrate is selected from the group consisting of corn starch, wheat starch, modified corn starch, a gum, whole, ground, cracked, milled, rolled, extruded, pelleted, defatted, dehydrated, solvent extracted, or other processed form of corn or wheat, and mixtures thereof; and

the amino acids, precursors or analogues of amino acids, complex protein sources, or proteins are selected from the group consisting of methionine, tryptophan, threonine, arginine, lysine, 2-hydroxy-4-(methylthio) butanoic acid, a salt of 2-hydroxy-4-(methylthio) butanoic acid, serum proteins, casein, soybean meal, fishmeal, meat meal, egg white, egg yolk, eggs without shells, and mixtures thereof.

63. The process of claim 62 wherein the gum is algin.

64. The process of claim 58 wherein the high moisture solid is made available to the poultry by placing the high moisture solid along with the poultry in a shipping container.

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